A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

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August 2012

# AN EXAMINATION OF VARIOUS SEGMENTS OF THE NITROGEN CYCLE IN DIVERSE AGRO-ECOSYSTEMS

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Cornell University 2012

Nitrogen is essential to plant growth and biomass production. Thus, it is often the primary focus in agricultural literature. The nitrogen cycle is complex in terrestrial systems mainly because it takes various forms - occurring in all chemical phases and mediated by both biotic and abiotic processes. The first chapter of this study examined the nitrogen cycle in its entirety as it relates to corn production in Iowa. Ultimately, this study was a proof-of-concept illustrating the possibility of adaptive nitrogen fertilization through the use of model simulation and improved VNIR and GIS technologies.

The next two chapters focused more specifically on gaseous nitrous oxide production in traditionally manured agroecosystems and potential interactions with carbon quality and quantity through the use of cover crops. Significant differences were found in the $N_2O$ emissions profiles between manured (M) and unmanured (NM) soils with a simultaneous increasing trend in emissions with increasing fertilizer applications. M soils produced 53-, 15.5- and 8.6-fold increases in $N_2O$ emissions over NM soils. Furthermore, on manured soils this study found that nitrous oxide emissions in cover crop treatments (winter rye and oats) showed no significant difference over a control plot during the early spring season. However, as ambient temperatures warmed, winter rye was found to decrease average $N_2O$ emissions by approximately 70% when compared to either the oat or fallow treatment. Additionally, nitrate concentrations found in

leachate at 55 cm were dramatically lower in rye plots, while the winter-killed oat showed no decrease in nitrate concentrations when compared to a control.

The final chapter concludes with a study on nitrogen mineralization in a Kenyan chronosequence amended with organic materials (Maize (MA) and Faidherbia albida (FA)). Mineralization patterns followed a similar trend in soils from a recent (New) and much older conversion (Old) to agriculture. Average mineralization in FA amended soils was 4.31% greater than the Control in the New soil and 0.75% in the Old soil during mineralization phases. In contrast, the MA treatment immobilized nitrogen for the majority of the study in both soils and to a greater extent in the more fertile New soil.

# BIOGRAPHICAL SKETCH

The second of three children, Chris was born in western South Dakota to parents Norman and Mary Graham. His father spent his career in elementary education as a teacher and principal while his mother worked in healthcare as a registered nurse and nurse practitioner. He has an older brother, Cary, and younger sister, Kelli. After high school, Chris attended the University of Portland in Portland, OR on an Air Force ROTC scholarship. He transferred to Arizona State University after his sophomore year where he graduated summa cum laude.

After graduation, Chris spent 11 months in Mauritius as part of a Fulbright Fellowship. He then attended Columbia University the following year and obtained a Masters of Public Administration with a concentration in Environmental Science and Policy. After a brief time working in Eastern Africa evaluating the effectiveness of agricultural water projects for the Rockefeller Foundation, Chris began his PhD studies at Cornell under the supervision of Dr. Harold van Es. Chris is married (Mikayla) and together they have one son (Elias) and two dogs (Zoe and Maya).

# ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my committee chairman, Harold van Es for his guidance, patience and counsel over the course of my dissertation. This body of work does not exist without his willingness to take a chance on me and his continued support throughout the process. I would also like to thank the rest of my dissertation committee, Dr. David Wolfe, Dr. Susan Riha and Dr. Jeff Melkonian, whom I relied on heavily for technical guidance and expertise. Additionally, I would like to thank the van Es lab, especially Bob Schindelbeck, for their input and support of my work.

Throughout the course of my study, I worked with several graduate students and support staff, whose help made the individual pieces of my dissertation happen. I would like to thank Dr. Joe Yavitt, Alexis Heinze and Kelly Hanley for access to and training on the gas chromatograph. I want to extend thanks to David Guerena for assistance with soil collection while in western Kenya as well as the staff at the Kisumu ICRAF. Further acknowledgement is required for help in gathering, processing and analyzing samples at Cornell, which includes Yandong Xue, Yuanun Zhu, Julius Blum and Marlene van Es.

Finally, the bulk of this study was funded by the U.S. Department of Agriculture Special Grant on Computational Agriculture, a collaborative program involving Cornell University's College of Agriculture and Life Sciences and its Center for Advanced Computing. Additional support was provided through a graduate student grant from the Northeast Region SARE program and the Richard Bradfield Award. And last, but certainly not least, I would like to thank my wife. Without her none of this would have been possible.

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# Chapter 1

# Improved Nitrogen and Energy Use Efficiency Using NIR Estimated Soil Organic Carbon and N Simulation Modeling

## 1.1 Abstract

In production agriculture, energy efficiency can be significantly improved by matching the crops N fertilizer needs and requirements. Current approaches for estimating optimum nitrogen fertilizer rates for maize (*Zea mays* L.) are generally based on regionalized mass balance equations or expected economic returns. However, N losses occur from dynamic and complex interactions among weather, soil organic matter mineralization and hydrology, crop water and N uptake, and management practices. This results in spatially and temporally variable fertilizer N needs. Studies have documented that early-season weather impacts changes between the inorganic and organic soil N pools, which contributes to variability in calculated maize economic optimum in-season N rate values. These interacting and complex spatio-temporal processes can be simulated by well-calibrated models.

This chapter discusses the integration of multiple data sources for improved estimates of maize N fertilizer needs. Data from an 11 ha field located in Iowa, USA will be used in this study. Equipment-mounted near-infrared spectroscopy was used to estimate soil organic carbon content at 1342 locations and kriged for 136 blocks. Soil texture and hydrology were determined from soil survey information. Using daily weather data and soil information, 24-year simulations were conducted using the Precision Nitrogen Management model to estimate late spring rootzone inorganic N content. This was combined with information on crop N uptake potential, mid-season N mineralization, and price ratio corrections to determine optimum sidedress N rates. Spatial and temporal variability in optimum N rate had a range of 60 kg $ha^{-1}$ and field scale maps were derived for the $10^{th}$ and $90^{th}$ percentile climate scenarios. This approach provides a

framework for integration of relevant spatio-temporal processes to create more precise and locally adapted N fertilizer recommendations for maize.

## 1.2. INTRODUCTION

### 1.2.1 Nitrogen Concerns

Applying unnecessary N fertilizer can reduce energy efficiency and profitability, and increase agricultural impacts on the environment (McIsaac et al., 2002). Maize, a $C_4$ plant, is physiologically more efficient at utilizing N (more yield per unit N accumulation) than most other major crops, which are generally $C_3$ plants (Greenwood et al., 1990). But paradoxically, maize production systems as a whole have low fertilizer N uptake and recovery efficiencies (RE). Through on-farm experiments in six North-Central US states, average RE was determined to be 37% with a standard deviation of 30% (Cassman et al., 2002). This suggests low nutrient use efficiency and both high and variable N losses to the environment.

Bergstrom (1987) and Randall et al. (1997) reported that in lysimeter studies maize had higher nitrate-N concentrations in leachate than less fertilized crops such as soybean (*Glycine max* L), wheat (*Triticum aestivum* L.), and perennial crops (e.g., alfalfa (*Medicago sativa* L.) and grasses. This was attributed to different fertilizer rates, fertilizer application schedules, timing of crop water and N uptake, and rooting depth. Intensive maize production areas therefore pose a risk for N losses to surface and groundwater systems and have become the focus of policy debates on addressing eutrophication and hypoxia concerns (Diaz and Rosenberg, 2008).

Energy consumption for N fertilizer production through the Haber-Bosch process and subsequent conversion of $NH_3$ to other forms of fertilizer ranges from 45 to 65 MJ per kg of N (Wang, 2007). Energy consumption associated with maize grain production can often account for up to 50% of the total energy use. Nitrous oxide losses from US agriculture are estimated at 377

pG $CO_2$ equivalent, which accounts for 57% of the total agricultural greenhouse warming potential associated with agriculture (US EPA, 2007). Based on several sources and assuming a mixture of N fertilizers, Snyder et al. (2009) estimated an overall greenhouse warming potential of 4 kg $CO_2$ $kg^{-1}$ N.

The environmental impacts of N fertilizer become a larger concern when N is applied in excess of the plant requirements. Studies by van Es et al. (2002) and Randall (2006) indicate that high nitrate leaching is primarily of concern when N is applied in excess of plant uptake. Similarly, Bouwman et al. (2002) determined that $N_2O$ emissions remain relatively constant in the range below the crop demand level (i.e., conservative fertilizer rates), but increase significantly when crop demand is exceeded. The precise estimation of optimum N fertilizer rates is therefore critical for both agronomic and environmental reasons (Ostergaard, 1997), but maize response to applied N is often highly variable and economically optimal N rates (EONR) may range from zero to 250 kg N $ha^{-1}$ (Scharf et al., 2006). Therefore, the estimation of the true EONR for a given specific location and growing season has remained elusive.

### 1.2.2 Estimating Optimum N Rates for Maize

In recent decades, the mass-balance approach has been the most widely used method for making N fertilizer recommendations (Stanford, 1973). It is generally based on a yield goal and associated N uptake, minus credits given for non-fertilizer N sources such as mineralized N from soil organic matter (SOM), preceding crops, and organic amendments. Several studies have documented, however, that the relationship between yield and EONR is very weak or non-existent for humid regions (Lory and Scharf, 2003; Vanotti and Bundy, 1994; Katsvairo et al., 2003, Sawyer et al., 2006a). For dryer regions, N response is more influenced by water limitations and associated unattained yield potential (Kim et al., 2008). The increased use of

yield monitors for site-specific yield measurement and grid-based soil sampling with SOM assessment have generated renewed interest in combining spatial yield data with the mass-balance approach to develop variable rate fertilizer application technology (Ferguson et al., 2002, Khosla et al., 2002). But Scharf et al. (2006) found that the EONR was more strongly related to spatial variability of soil N factors than crop N requirements. In humid regions, yield patterns themselves are highly variable from year to year depending on weather conditions (Katsvairo et al., 2003; Kahabka et al., 2004), and any mass balance approach to N fertilizer recommendations would pose the challenging task of predicting yields in the early growing season.

Several leading US maize-producing states have adopted the maximum return to N (MRTN) approach (Sawyer et al., 2006a), which largely abandons the mass-balance method. It provides highly generalized recommendations based on multi-year and multi-location field trials, curve-fitting, and economic analyses (Vanotti and Bundy, 1994). Adjustments based on realistic yield expectation are sometimes encouraged. However, owing to its generalization over large areas, soil types and across seasons, it is not adaptive to local conditions and does not address or account for spatial and temporal processes that affect N availability to maize.

### 1.2.3 Temporal and Spatial N Dynamics

EONR is affected by spatial and temporal processes, and multiple N sources may contribute to the pool of N available for maize N uptake. Approximately 190 kg N $ha^{-1}$ is needed to produce 10 Mg $ha^{-1}$ of maize grain in Nebraska, according to Cassman et al. (2002). Mineralization of SOM in central United States can range from 50 to 250 kg N $ha^{-1}$, in eastern USA mineralization is slightly lower and averages 80 kg N $ha^{-1}$ (Ketterings et al., 2003). Lobell (2007) reported that more precise N management and potentially significant long-term reductions in N rates for maize production with no yield penalty could be obtained if site-specific

estimates of SOM mineralization can be made. The difference between the crop requirement (which itself is affected by seasonal environmental stresses) and the soil supply is ideally provided by fertilizer supplements. But the precise estimation of this difference and the associated fertilizer use efficiency remains a challenge due to numerous sources of variability.

Early season weather, particularly precipitation, has been highly correlated with seasonal variation in optimum fertilizer N rates and nitrate-N losses from crop fields (Balkcom et al., 2003; Mitsch et al., 2001). Sogbedji et al. (2001c) found that growing seasons with excessive wetness in late spring showed lower maize yields and higher EONRs than other years, with an estimated range of 90 kg $ha^{-1}$. In normal years in humid, temperate climates, accumulation of mineral N in the root zone from SOM mineralization may contribute about half of the required maize N (Fig.1). The crop N uptake curve lags behind the organic N mineralization curve until the rapid uptake phase during the mid-vegetative period.

During the late spring, high quantities of soil mineral N (SMN) reside in the soil profile, mostly in the nitrate form that is subject to loss. If excessive rainfall occurs during this critical period, significant N losses may occur from leaching or denitrification (with warm soil, Fig. 1). SMN accumulation is generally higher for soils high in organic matter, but this may be subject to losses as well (Kay et al., 2006). Losses are also affected by the accumulation of heat units over the first months of a growing season. In cool springs, N mineralization is slow, and the accumulation and subsequent loss of SMN is smaller when excessive wetness occurs (Fig. 1). Also, the date of planting affects the length of the critical period before the rapid growth period.

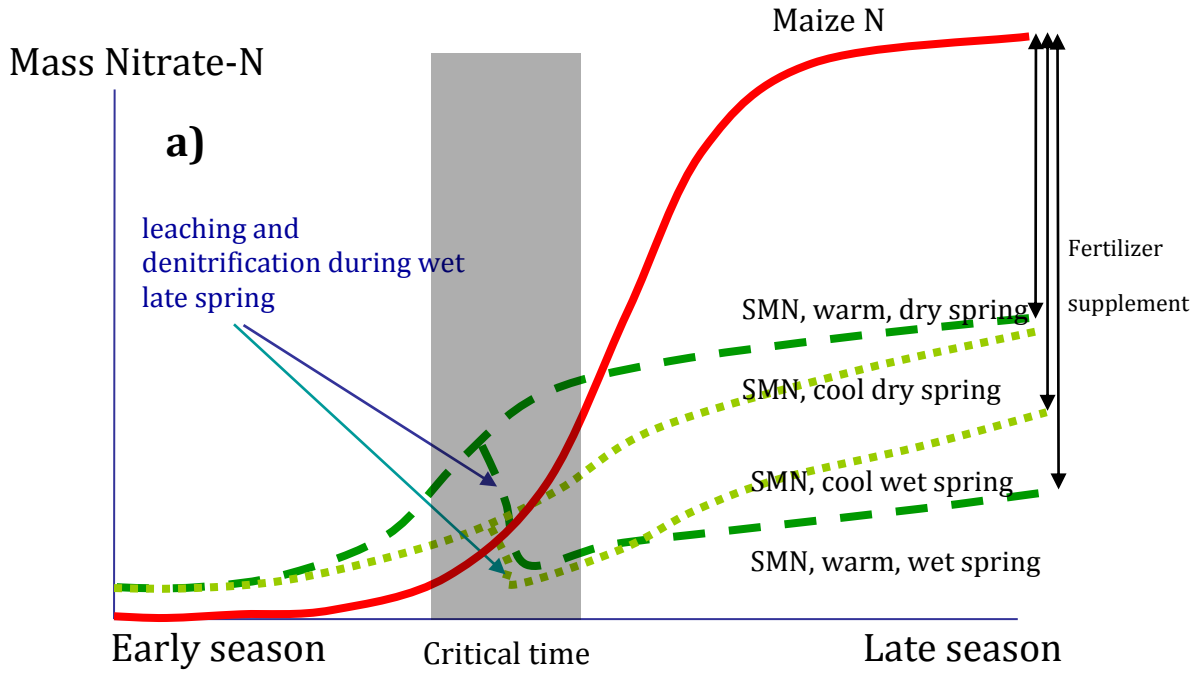


**Figure 1.1**. Conceptual gains and losses of soil mineral N (SMN) and crop N over a growing season for a low soil N system. Broken and dotted lines represent SMN accumulations; solid line depicts maize N uptake (after van Es et al., 2007).

*Early*-season weather appears to be the strongest determinant for seasonal N availability for the crop, but m*id*- and *late*-season weather, especially the occurrence of drought, may also affect yields and create unattained yield potential. When the crop is well fertilized this tends to result in high residual N levels at the end of the growing season that are subject to environmental losses.

Several spatial factors impact EONR as well. Sogbedji et al. (2001c) determined that spatial effects were not consistent from year to year and that they interacted with weather. For example, poorly drained areas had a wider range of annual EONR values (high in wet springs and low in dry springs) than well-drained areas. Similarly, Kay et al. (2006) determined that organic matter content (a spatially variable N source) and early-season rainfall (driving force for

N losses) were the main predictors of seasonal N availability, but they strongly interacted in a nonlinear manner.

In all, optimum N fertilizer rates vary greatly and primarily depend on (i) the amount of readily mineralizable nitrogen in the soil and the mineralization pattern during the growing season, (ii) early growing season N losses related to the occurrence and timing of excess wetness and high soil temperatures during those times of saturation, and (iii) the occurrence of drought during the mid and late season, resulting in unattained yield potential. Static methods for determining fertilizer rates, including current mass-balance and MRTN approaches, have limited success because they neglect temporal and spatial dynamics in soil N. Use of static fertilizer rates generally results in excessive fertilization in years with dry springs and summers, and inadequate fertilization in years with high N losses from wet spring soil conditions.

In many cases, especially when fertilizer to crop price ratios are low, farmers opt to use higher N fertilizer rates (insurance fertilizer) to avoid the risk of yield loss in the event of a wet season. In the majority of years this results in excessive fertilizer application, unnecessary expense, and increased N losses that adversely impact the environment (Sogbedji et al. 2000a; Randall, 2006).

Improving the current in-season N recommendations for maize is critical to the credibility of fertility recommendation systems, and increased N use efficiency is expected to reduce residual soil N that may be lost to the environment (van Es et al., 2002). This chapter demonstrates a locally adaptive approach that accounts for several sources of spatial and temporal variability, notably variations in weather and SOM, to obtain more precise and site-specific N fertilizer recommendations.

## 1.3. OBJECTIVES, APPROACH, AND TECHNOLOGIES

### 1.3.1 Objectives and Approach

Our objective is to establish a framework for site-specific and season-specific nitrogen recommendations for maize production in the midwestern USA. We are accomplishing this through the integration of several technologies, including a vehicle-mounted near-infrared spectrometer that is providing information for estimating soil organic carbon, soil survey information, weather (climate) data, a deterministic N simulation model, and a geographic information system. Our study site is situated in east-central Iowa (Bremer County; 42° 45' 25" N, 92° 32' 13" W), and consists of gently sloping loam soils across approximately 11 hectares. We used a site and season-specific mass balance approach in that the various components of the nitrogen mass balance were estimated in a spatially and temporally explicit manner and were allowed to interact.

### 1.3.2 Estimating Soil Organic Carbon with Near-Infrared Spectroscopy

Mineralized organic N is an important source of N for a maize crop, but may have high spatial variability across single fields due to soil forming and erosion patterns. Mapping soil fertility indicators and quantifying soil parameters that control soil processes are important for site-specific soil management. Large numbers of samples must be collected and analyzed in order to capture this spatial variability and adequately estimate soil properties. Conventional methods may be expensive and require large amounts of labor and chemicals for performing these tasks (Viscarra Rossel and McBratney, 1998).

Near infrared (NIR) reflectance spectroscopy is a low-cost method that can be used to substitute or complement traditional soil characterization methods. It measures soil constituents that have unique absorption features in this wavelength region due to overtones related to

stretching and bending vibrations in molecular bonds such as C-C, C-H, N-H and O-H (Dalal and Henry, 1986). Once calibrated, the methodology can be used to predict multiple soil characteristics simultaneously and explain within-field spatial variability. Chang et al. (2001) and Reeves et al. (2002) reported successful predictions ($R^2$> 0.80) for several properties including total organic carbon and nitrogen (g $kg^{-1}$), which are important properties for precise N management.

**1.3.3 Using Models for N management**

More precise management of N under maize in humid regions requires the explicit consideration of interacting factors that vary in both space (site-specific) and time (primarily as defined by variation in weather conditions). In humid regions, crop N requirements for maize cannot be accurately predicted at the beginning of the growing season (even less so during the previous fall), because one of the main determining factors (spring weather) is still undetermined at that time. Even slow-release or nitrification-inhibition technology and early season soil testing can only achieve limited accuracy, because a large part of the maize N needs are derived from organic N mineralization that is affected by early-season weather factors.

Environmental information systems and simulation models effectively allow for incorporation of both temporal and spatial processes related to N dynamics. This approach can take advantage of increasingly sophisticated environmental databases (e.g., radar-based precipitation estimates); Melkonian et al., 2007) that can be accessed as input information for dynamic soil and plant models to estimate crop growth and soil N dynamics, and provide more precise estimates of seasonal crop N needs (Olness et al., 1998; van Alphen and Stoorvogel, 2000). If these models are well-calibrated and tested, they can provide information to growers to

adjust in-season N applications to more precisely match crop N demand (Kersebaum, 1995; Smith et al., 1997).

The Precision Nitrogen Management (PNM) model (Melkonian et al., 2005; Melkonian et al., 2007) was developed to track soil and crop N flows in maize cropping systems. Critical outputs of the PNM model are simulated values of mineralized N and losses through leaching, denitrification, and volatilization, as well as crop N uptake and biomass (vegetative and grain) accumulation.

The model has two components: LEACHN, the N (and phosphorus) module of LEACHM (Hutson, 2003) and a maize N uptake, growth and yield model (Sinclair and Muchow, 1995). LEACHN is a process-based, one-dimensional model that simulates water and solute transport, and chemical and biological N transformations in the unsaturated soil zone (Hutson, 2003). Flows between different pools of C and N are simulated in each soil segment as well as on the soil surface. LEACHN is well suited for simulating soil N processes and has been extensively used and tested in several studies (Jabro et al., 1994; Jemison et al., 1994a,b; Lotse et al., 1992; Sogbedji et al., 2001a,b; Sogbedji et al., 2006).

The rate constants in the equations describing nitrification, denitrification, manure mineralization and plant residue mineralization were calibrated based on multi-year, replicated field experiments (Sogbedji et al., 2000b; van Es et al., 2006). In the PNM model, SOM mineralization is simulated using two rate constants instead of one as in LEACHN: a higher rate constant for early season SOM mineralization (up to July 15) and a lower rate constant for later in the season, based on studies by Dharmakeerthi et al. (2005).

The crop component of the PNM model is based on a maize N uptake, growth and yield model (Sinclair and Muchow, 1995). The subroutines of the maize N uptake, growth, and yield model

incorporate the effects of temperature, solar radiation, water supply and parameters influencing the crop N budget during the three major phases of maize development: vegetative growth, anthesis, and grain fill (Muchow and Sinclair, 1991; Muchow et al., 1990; Sinclair and Amir, 1992; Sinclair and Muchow, 1995).

### 1.4 Methodology

#### 1.4.1 Mass Balance Inputs

To calculate the site and season specific N-fertilizer recommendations we used the basic mass balance approach (Stanford, 1973, Lory and Scharf, 2003). As Stanford (1973) notes, the mass balance approach as it was originally formulated does not necessarily encompass the dynamic nature of the ecological system controlling nitrogen. We postulate that the concerns with the mass balance approach are primarily the result of unaccounted for site and season specific conditions (esp. seasonal variations in rainfall), thereby masking the inherent scientific validity of the methodology. In the approach discussed below we explicitly incorporate the spatial and temporal sources of variability (soil types, drainage class, organic matter content, weather, and crop management) as well as their interaction in the estimation of maize N fertilizer needs, assuming that the timing of fertilizer addition is optimized and occurs as sidedress or topdress in the late spring - for our case presumed to be soon after 15 June. A modified version of the mass balance equation was used:

$$Nrec_i = Nupt_i - Nrz\_pre15jun_i - Nrz\_post15jun_i - Npr\_corr_i \quad [1]$$

where $Nrec_i$ is the sidedress N recommendation for a management unit i, $Nupt_i$ is the total crop N uptake, $Nrz\_pre15jun_i$ is the total rootzone N on 15 June, $Nrz\_post15jun_i$ is the estimated N

mineralized from 15 June to harvest, and $Npr\_corr_I$ is a correction between the estimated maximum yield and the economically optimum yield based on the fertilizer-to-grain price ratio. These recommendations were made for each of 136 blocks within the field for two climate years and two price ratios. The following sections describe how the input data for the mass balance equation were obtained and the recommendations derived.

### 1.4.2 Yield Goal and Estimated N Uptake

The yield goal was fixed at 11.0 Mg $ha^{-1}$ for the purpose of this research, which was in line with the estimates from the PNM model (Table 1). $Nupt_i$ (kg $ha^{-1}$) was estimated by multiplying the yield goal by 21.4, which is a standard assumption for maize production (Kim et al., 2008).

**Table 1**. Maximum yield and optimum N fertilizer rates at maximum yield and 5.6 and 11.2 fertilizer-to-grain price ratios.

| | Max Yield | N Rate at Maximum Yield | EONR at 5.6 Price Ratio | EONR at 11.2 Price Ratio |
|---|---|---|---|---|
| | Mg $ha^{-1}$ | ---------- | kg $ha^{-1}$ | ---------- |
| **Floyd Loam - 1.5% OC** | 10.92 | 190 | 175 | 163 |
| **Floyd Loam - 2% OC** | 10.98 | 180 | 170 | 154 |
| **Floyd Loam - 2.5% OC** | 11.22 | 181 | 168 | 151 |

### 1.4.3 Soil Survey Data

The PNM model requires basic soil information including textural class, drainage class, and organic C (or organic matter) content by depth to 1 m. Other than organic C, basic soils data were derived from the Natural Resources Conservation Service Soil Survey database through the

web soil survey portal (http://websoilsurvey.nrcs.usda.gov). Six soil types were mapped for the field (Fig. 2), mostly Floyd loam (fine-loamy, mixed, superactive, mesic Aquic Pachic Hapludoll) and geographically associated soils (Clyde silty clay loam, Kenyon loam, Ostrander loam, Waukee loam, and Dickinson fine sandy loam). Slope classes included A: 0-to-2 percent; B: 2-to-5 percent; and C: 5-to-9 percent. Soil profile data including drainage class and textural class by horizon were derived from the soil survey report (Camp, 2009). Soil survey data were adapted to quantitative data by depth layer as close as possible, which in some cases involved interpolation. The soil survey information was digitized and entered into ArcGIS (ESRI, Redlands, CA) software.


**Figure 1.2** Study site and soil map (from USDA-NRCS Web Soil Survey).

### 1.4.4. Soil Organic Carbon from NIR Reflectance Spectroscopy

The field distribution of soil organic C content was obtained through the use of a vehicle-mounted visible and near infrared (VIS-NIR) Reflectance Spectroscopy unit that was located in a soil-engaged shank (Veris Technologies, Salina, KS). Data from this field and seven nearby fields were used to calibrate the NIR estimates (with visible wavelengths deleted) with laboratory measured samples using partial least squares regression with an optimum number of principal components (5) using The Unscrambler v 8.0 software (CAMO Software AS, Oslo Norway). Approximately 20 GPS registered soil samples were collected from each field immediately after the field mobile NIR spectrometer passed the sample location. Thus spectra collected real-time in the field were available for each of the calibration samples. Conservative leave-one-field-out calibrations were based on truncated first difference spectra in the wavelength range of 1002-1681 nm. This yielded a calibration curve with a coefficient of determination of 0.75 (Fig. 3).

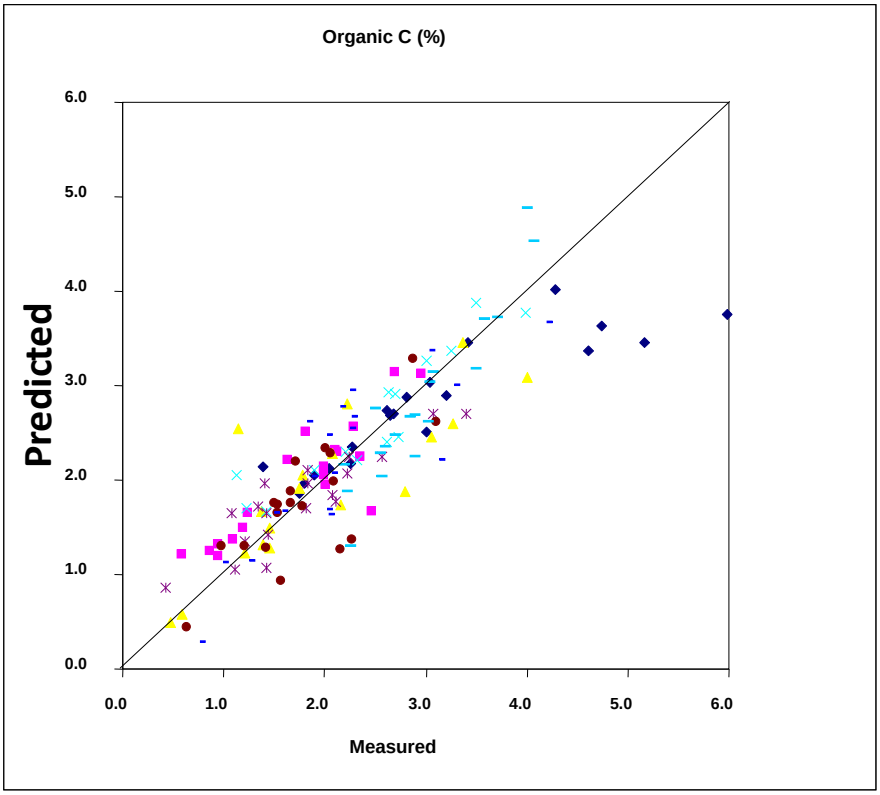


**Figure 3**. Correlation between laboratory-measured and NIR-estimated organic C contents obtained using leave-one-field-out cross validation for eight fields in East-Central Iowa ($R^2$=0.78; RPD=2.1). Different symbols represent different fields.

The field mobile NIR system was used to estimate organic C for 1302 locations within the field, which were consolidated into 136 30x30 m management units using block kriging based on spherical variograms and examination of quantile-quantile (QQ) plots in the ArcGIS Geostatistical_Analyst tool (Fig. 4). These organic C estimates, combined with the soil survey information, provided the basic soil inputs for the N simulations.

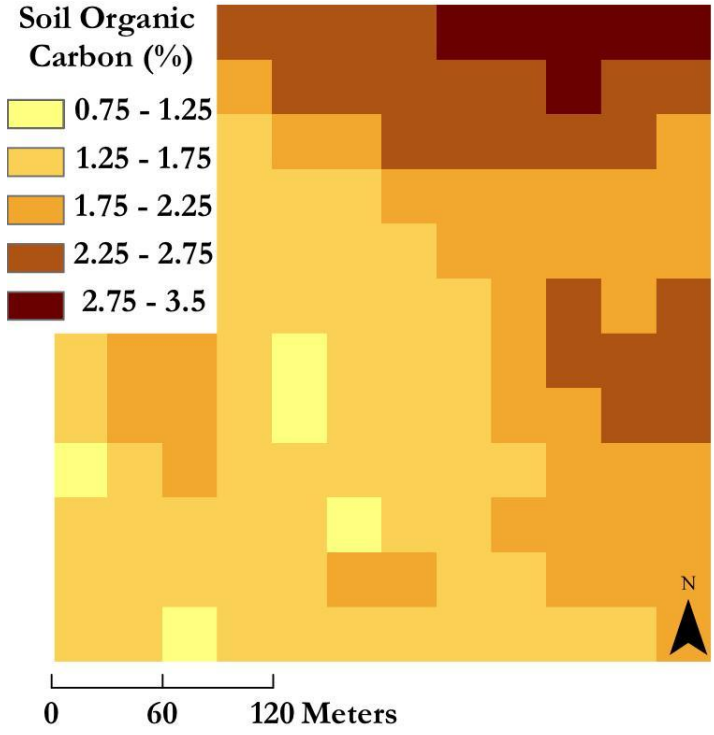


**Figure 4**. Soil organic C content distribution across the study site.

### 1.4.5 PNM Model Simulations

After the soil data were entered into the model, multi-year PNM simulations of soil and crop N dynamics were performed for 24 climate years (1985 to 2008) based on observations from a nearby weather station (Dumont, IA). We simulated a continuous-maize cropping system under a plow-till system. N was applied as a 32% solution of urea and ammonium nitrate (UAN32) with 22.42 kg $ha^{-1}$ applied at planting on May 1$^{st}$ each year and an additional sidedress application of 145.73 kg/ha (130 lbs/ac) applied on June 15$^{th}$. Simulations were executed at the Cornell Center for Advanced Computing for each of the 136 management units of the field based on the site-specific soil information.

## 1.5 Results

### 1.5.1 Rootzone N in Early Season

The PNM simulations yielded estimates of the amount of mineral rootzone N on 15 June ($Nrz\_pre15jun_i$ ) for each of the 136 management units and each of the 24 simulation years. Figure 5 shows the distribution of the rootzone N on 15 June for the $10^{th}$ and $90^{th}$ percentile of the 24 simulation years, the former generally associated with years of wet spring weather. The spatial patterns in both years are correlated to the distribution of organic C content (Fig. 4), indicating greater N mineralization with higher soil organic matter levels. Also, there is a range of 40 kg $ha^{-1}$ among the locations and years.

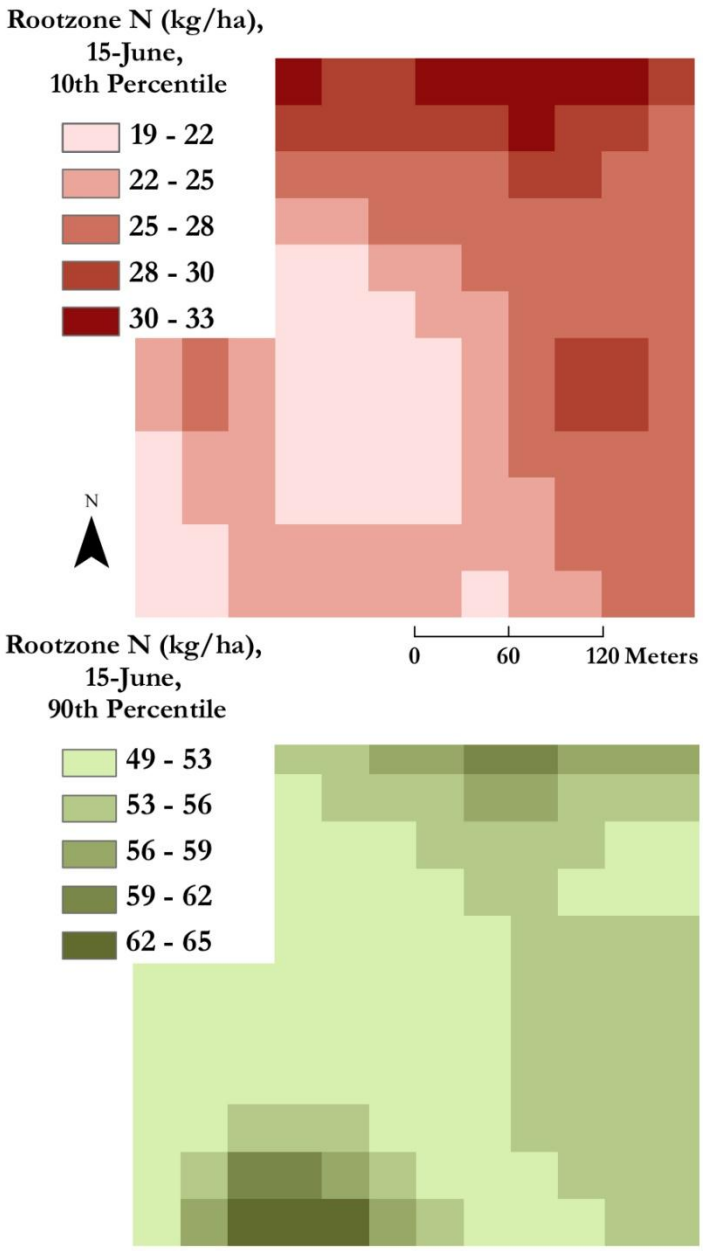


**Figure 5.** The 10th and 90th percentile simulated rootzone N at 15 June based on 24 climate years.

### 1.5.2 Rootzone N Mineralization in Late Season

The model simulations also provided estimates for post-sidedress mineralization amounts (June 16 to harvest; $Nrz\_post15jun_i$). This represents the soil N that is mineralized in the mid and late growing season that contributes to crop N availability. Since this contribution occurs after the time of fertilizer application, it cannot be estimated based on the conditions for a specific

growing season. However, it can be assumed that this N release is not subject to leaching or denitrification losses due to high crop transpiration amounts during this time period (primarily due to the large leaf area in mid and late season). We therefore generated site-specific estimates for each of the 136 management units based on the average mineralization during the post 15 June period (Fig. 6). The N contributions for mid and late season mineralization were estimated to range from 20 to 38 kg $ha^{-1}$ for the different locations in the field, following mostly the distribution in soil organic C content (Fig. 4).

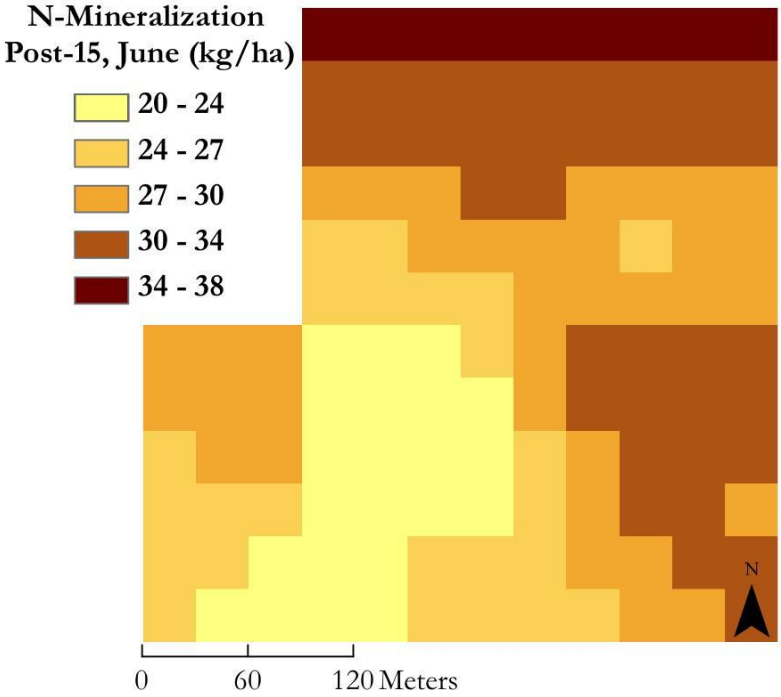


**Figure 6**. Average simulated N contributed during mid and late season (15 June and harvest).

### 1.5.3 Price-Ratio Correction

Maize response to nitrogen is a nonlinear process, and the maximum yield is generally not the economically optimum yield. In his original assessment, Stanford (1973) noted that the final 10% of yield required up to half the total N applied. The EONR accounts for this fact by

calculating the marginal return of N (Nafziger et al., 2004), which effectively calculates the rate at which fertilizer application no longer returns a profit. This generally depends on the cost of fertilizer relative to the return rate (price) of the grain, which is expressed as the price ratio [\$ $kg^{-1}$ of N per \$ $kg^{-1}$ of maize grain). These price ratios vary with commodity prices, but have nevertheless not changed much over the long term, because maize grain and fertilizer prices tend to rise and fall together (Bruulsema and Murrell, 2008).

Estimating the EONR was performed using the PNM model. Naturally, the ratio of the price of fertilizer to the price of maize will alter the rate at which maximum profit can be attained where the larger ratio (i.e., high fertilizer cost relative to grain price) decreases the economic optimum rate. We conducted a separate set of 24-year PNM simulations to estimate appropriate price ratio corrections, $Npr\_corr_i$ in Eq. [1], for the dominant Floyd loam soil at organic C levels of 15, 2.0, 2.5, and 3.0%. Simulations were performed for N sidedress rates of 0, 25, 50, 75, 100, 125, 150, 175, and 200 kg N $ha^{-1}$ to define the N response curve, which was subsequently modeled with a quadratic equation using Microsoft Excel (Microft Corp., Redmond, WA). This allowed for the quantification of the maximum yield as well as the economic optimum yield at two price ratios, 5.6 and 11.2 (Table 1). For the 5.6 price ratio the EONR was approximately 10 to 15 kg $ha^{-1}$ lower than the rate at maximum yield; at the 11.2 price ratio it was 26 to 30 kg $ha^{-1}$ lower. The price ratio effect was very similar among soil organic C levels and a soil-averaged $Npr\_corr_i$ value (corrected as yield above zero) was therefore used for each price ratio, and each was uniformly applied to all management units in the field.

**1.5.4 N Recommendations**

After having obtained all input data for Eq. [1],the N fertilizer recommendation, $Nrec_i$ can be calculated for each of the 136 management units in the field. This data set is available at

data chapter 18. Estimates were made for 2x2 factorial combinations: the $10^{th}$ and $90^{th}$ percentile of the distribution of simulated 15 June N mineralization for the 24 climate years, and the 5.6 and 11.2 fertilizer-to-maize grain price ratios. Figure 7 shows the maps of recommended N fertilizer rates.

The higher recommended rates are in the areas with low organic C content (Fig. 4) and N mineralization potential (Figs. 6 and 7). The recommended rates ranged from 149 to 179 for the $10^{th}$ percentile at the 5.6 price ratio, and were reduced by approximately 30 kg $ha^{-1}$ (119 to 146) for the $90^{th}$ percentile, which represents years with dry early growing seasons that experience little N loss from excessive rainfall. The effect of the price ratio reduces the recommended N rate by 17 kg $ha^{-1}$ for the 11.2 compared to the 5.6 price ratio.

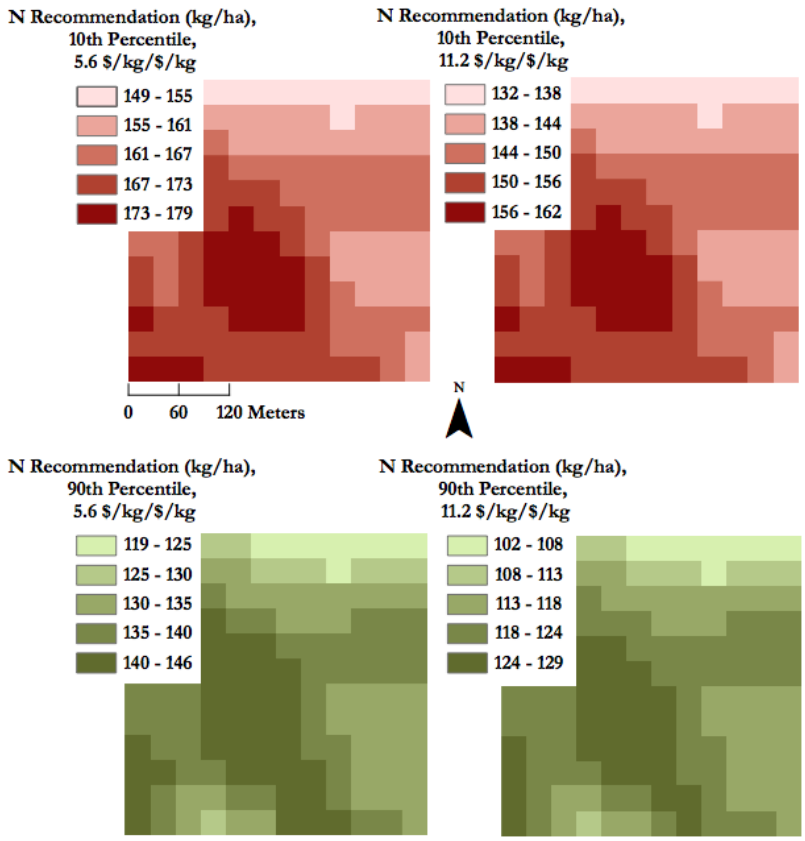


**Figure 7.** Recommended nitrogen fertilizer rates at 5.6 and 11.2 price ratios for the 10 and 90th percentiles of the distribution of rootzone N on 15 June.

## 1.6 Discussion and Conclusion

The above process describes a framework for improved (more precise and locally-adapted) estimation of economically optimum fertilizer N rates for late spring applications. We used 24-year climate data to gain a better understanding of the effects of early-season weather and we used NIR-based estimates of soil organic C and soil survey information as inputs for assessing the spatial variability component. These technologies are currently available and

implementation and testing of this approach is therefore feasible. For a typical management implementation, the simulations would be done for only the present growing season.

In our analysis, the ranges associated with spatial (primarily soil-related) variability and temporal (primarily weather-related) variability were estimated to be approximately the same, 30 kg $ha^{-1}$, with little interaction. This implies that EONR for any location and any year may vary by up to 60 kg N $ha^{-1}$ (Fig. 7) for a given price ratio. This could provide significant savings to a farmer and reductions in environmental losses. The benefits of using seasonally adapted N rates will be enhanced if the supplemental N applications occur somewhat later (e.g., early July) in the growing and can incorporate longer weather records. The approach can also be used with conservative at-planting N fertilizer applications and model-based supplemental rates in the years with wet springs.

The spatial variability may be somewhat higher than predicted by our model. The PNM model performs one-dimensional simulations of the soil-crop system, and cannot account for three-dimensional redistribution of water in the landscape. This means that imperfect drainage in lower landscape positions may be accentuated and the N losses are greater in those locations in wet early growing seasons.

The effort involved in developing the N fertilizer recommendations may appear excessive, but the framework mostly involves one-time investments. With the increased availability of NIR spectroscopy for field scale soil assessment - or alternatively the more conventional grid-based soil analyses or Order 1 soil surveys (Chang et al., 2004) - many farmers are willing to invest in the characterization of soils at the sub-field scale if they can more profitably manage crop inputs. This one-time investment can then be entered into a GIS and used for multiple years of soil and crop management applications. The N recommendation

system can be built into a GIS, but requires an infrastructure to perform dynamic simulations in real-time. The Adapt-N tool (http://adapt-N.eas.cornell.edu) is currently available for such purposes, where the PNM model can be run based on stakeholder inputs and up-to-date high-resolution climate data (Melkonian et al., 2007).

Our approach may be enhanced with several additional data sources. We assumed a fixed yield potential for the entire field. Yield monitor data can be used to provide more site-specific yield estimates, which can then be entered into Eq. [1]. However, yield patterns themselves may vary among growing seasons (Katsvairo et al., 2003; Kahabka et al., 2004; Chang et al., 2004) and are still mostly undefined by late spring, posing a challenge for management purposes.

In conclusion, the EONR for maize in any field is not a fixed quantity, but varies as a result of several interacting spatial and temporal factors. The most significant among those are early-season weather (precipitation and temperature), N mineralization from organic sources, and crop development. Most current N fertilizer recommendation systems ignore these dynamic processes, which limits their capacity to precisely manage N. We presented a framework for improved estimation of EONR for maize that accounts for these spatial and temporal processes. Gains from this approach appear to be considerable, but field verification will be required for future adoption.

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# Chapter 2

# Spring nitrous oxide emissions and nitrate leaching losses under winter cover crops on a manured field

## 2.1 Abstract

Increased animal production and concomitant land application of manure create conditions conducive for significant environmental losses of manure-N. Scavenger cover crops have shown some promise in mitigating off-field losses. In this study, we tested the effects of oat (*Avena sativa*) and winter rye (*Secale cereal*) cover crops on N losses in soils with a history of manure application during the volatile spring season. Undisturbed cores were taken from replicated plots planted to oats, winter rye or left fallow at three points during the spring season. After a simulated rainfall, emissions were collected for a subsequent 96-hour incubation period.

Nitrous oxide emissions in cover crop treatments showed no significant difference over a control plot during the early spring season. However, as ambient temperatures warmed, winter rye was found to decrease average $N_2O$ emissions by approximately 70% when compared to either the oat or fallow treatment. Furthermore, nitrate concentrations found in leachate at 55 cm were dramatically lower in rye plots, while the winter-killed oat showed no decrease in nitrate leaching when compared to a control. These contrasts in oat and rye treatments were likely the result of the re-growth of the rye during the spring sampling period. Stimulation of N losses in manured fields could in fact be hastened during the spring season with the use of oats as a fall cover crop, while rye appears to reduce environmental N losses during the same period.

## 2.2 Introduction

### *2.2.1 Manure and N Losses*

Approximately 140 Tg of manure-N is applied to arable lands each year, which has

roughly doubled since World War II (Davidson, 2009). Application of manure often includes applying nutrients in incompatible ratios with those for crop uptake (Edmeades, 2003). Due to the simple issue of disposal, accumulation of macronutrients (particularly N and P) is a likely result. Furthermore, manured agricultural systems often combine low N-use efficiency with readily available carbon for high N losses (Oenema et al., 2010).

With the high variability of manure composition, the extent of available N and C is inconsistent in these systems. In a study of manure mineralization dynamics, van Kessel et al. (2000) found that compounds such as urea, simple peptides and amino acids, proteins, ruminal bacteria and colonic cells stimulated faster mineralization while other, more recalcitrant compounds, such as ADF, NDF and lignin resist decomposition and mobilization. Additionally, as nutrients are mobilized, they are subject to rapid adsorption by the soil matrix (Angers et al., 2006). In a study of fall-applied manure, Eghball (2000) found that 11% of composted manure and 21% of non-composted manure N was mineralized during the following year. Consequently, the temporal component of this system will have a large effect on nutrient losses.

Spring thaw in temperate climates is a period of potential high N losses. Christensen and Tiedje (1990) found $N_2O$ production to be far higher during the thaw period than in any other period tested throughout the year. Further research has produced similar results from cores subjected to freeze/thaw cycles, which suggests a possible mechanism for physical disruption of aggregates to promote the production of $N_2O$ (Christensen and Tiedje, 1990, Christensen and Christensen, 1991, van Bochove et al., 2000, Muller et al., 2002). Using $^{15}N$ techniques, Wagner-Riddle et al. (2008) found that high emissions were due to newly produced gas as opposed to older $N_2O$ that might have been trapped deeper in the soil profile. Using frozen and unfrozen cores with a straw amendment, Christensen and Christensen (1991) found that extractable carbon

increased after a thaw period and then returned to levels similar to the unfrozen treatment on subsequent freezing while maintaining significantly higher denitrification levels. This suggests that while freezing and thawing may produce available carbon through dead microbial biomass, additional carbon is liberated from previously sequestered fractions in the soil. Thus $N_2O$ production in the spring is greatly facilitated by the amount and quality of carbon present in the soil before the winter-freeze period.

Nitrogen losses in the spring are especially acute in manured systems. In the denitrification process, high levels of organic carbon in manure correlate with increased $N_2O$ emissions (Beauchamp and Paul, 1989, Clemens and Huschka, 2001, Miller et al., 2009). The incorporation of manure in the fall promotes $N_2O$ emissions with as much as 65% of yearly emissions occurring in the spring thaw period (Wagner-Riddle et al, 1997). Additionally, leaching losses of $NO_3$-N may be considerable, particularly when fall-applied early and in warm, wet autumns (van Es et al., 2006).

Beckwith et al. (1998) found that applications of various manures applied between September and November significantly increased leaching over a control with as much as 20% leaching loss as a percentage of total applied. Similarly, Smith et al. (2002) found average leaching losses of approximately 15% of applied manure-N with high CV values ranging from 2 to 53%. Furthermore, nitrate leached into groundwater is subject to rapid degassing as $N_2O$ when re-exposed to the atmosphere after discharge into drainage ways (Reay et al., 2003). The current emissions factor for this indirect pathway under IPCC guidelines is 0.0075 kg $N_2O$-N $kg^{-1}$ per kg N leaching/runoff with a considerable range of uncertainty (IPCC, 2006).

### *2.2.2 Cover Crops*

Winter cover crops are increasingly adopted for both N-fixing and N-foraging purposes, as well as their ability to suppress weeds, reduce erosion and improve water-holding capacity (Fageria, 2007; Kaspar et al., 2007; Meisinger et al., 1991). Cover crops may interact with soil nitrogen cycle dynamics, potentially serving as an additional C source to fuel nitrate reduction or, conversely, by immobilizing residual N making it unavailable for chemical and physical soil processes. Cover crop utilization of N, like any other link in the nitrogen cycle, is highly variable and subject to prevailing environmental conditions and indigenous soil properties (Thorup-Kristensen et al., 2003).

Various cover crops can take up and release N at different times and rates, which makes effects difficult to study and sometimes contradicting. Jarecki et al. (2009) found contrasting results in laboratory and field experiments on denitrification. Their research concluded that a rye cover crop reduced $N_2O$ emissions in a microcosm experiment with swine manure additions. However, in field studies, rye and oat cover crops had no effect on emissions. On the other hand, Sauer et al. (2009) investigated the interacting effects of a rye cover crop with poultry manure in a bermudagrass pasture and found that rye reduced denitrification losses in the spring, which they attributed to a reduced soil $NO_3$ -N pool. Moreover, N uptake by the succeeding crop may be erratic. Ball et al. (2005) found residue-derived-N content in grain to vary according to climatic factors. In wet years N was a limiting factor and cover crops improved yields. This uptake is likely to be variable even within a growing season, which would likely contribute to field-scale variability in subsequent trial periods.

The objective of this study was to determine the impact of two cover crops - rye and oat- on nitrogen flows in a manured agroecosystem during the volatile spring season. Rye and oats

were selected as treatments due to their popularity in the northeastern USA and also for their physiological disparity in winter tolerance and how this affects carbon and nitrogen cycling. Manured plots are expected to have high baseline $N_2O$ emissions and additional losses through $NO_3$ leaching. The aforementioned cover crops may immobilize some of the residual N, thereby reducing losses. However, because oats winter-kill, we hypothesize that the N taken up by the crop will simply be lost in the early spring, through enhanced $N_2O$ emissions supported by labile carbon from the decaying oat crop and through leaching. Rye, on the other hand, will re-establish growth in the spring, which we predict will continue N immobilization and reduce environmental losses. Thus, the objectives of this study were to examine denitrification potential after a simulated heavy rainfall to detect differences in emission patterns from the different cover crops. Furthermore, this study monitored nitrate concentrations in soil pore water at 55 cm. to assess nitrate leaching out of the effective root zone of each treatment.

## 2.3 Materials and Methods

### *2.3.1 Study-Site*

This study was conducted on a working dairy farm located in Lansing, NY, USA (42°35' N, 76°31'W; 264 m a.s.l.) using a field with a history of manure application. The soil at the research site is an Ovid silt-loam (fine-loamy, mixed, active, mesic Aeric Endoaqualf) with average surface soil texture measured as 330 g $kg^{-1}$ sand, 550 g $kg^{-1}$ silt and 120 g $kg^{-1}$ clay determined using procedures described by Kettler et al. (2001). Average organic matter content in the surface soil was 40 g $kg^{-1}$ soil, determined gravimetrically by loss on ignition and pH 7.1, determined in a 1:1 water slurry. During the past three years, manure was applied on 10 April 2008, 5 October 2009 and 15 April 2010 (final application before study commencement) at rates of 64, 75 and 43 $m^3$ $ha^{-1}$, respectively. The average N content for liquid manure on the farm is

approximately 2.5 kg $m^{-3}$.

Primary field sampling was conducted during three separate events in April and May of 2011 when the normal temperature ranges from 1 to 13 °C and 6 to 20°C respectively. Normal total precipitation for these months is typically 84 mm in April and 82 mm in May. Over the experimentation period, however, April and May rainfall were about twice the average (188 and 157 mm, respectively).

### *2.3.2 Cover Crops*

Winter rye (*Secale cereale* L.) and oat (*Avena sativa* L.) were broadcast seeded on 24 September 2010 in a 3x4 spatially-balanced, complete block design (van Es et al., 2007) at a rate of 112 kg $ha^{-1}$ (Fig. 1). Along with control plots, each cover crop treatment was replicated four times for a total of twelve blocks. Quadrats of rye and oats were subsequently harvested on 3 December 2010 and analyzed for N uptake by dry combustion using a Europa ANCA-GSL CN auto-analyzer (PDZ Europa Ltd., Sandbach, UK). The rye plots were harvested two additional times in the following Spring on 22 April and 26 May. Roots were harvested to a depth of 15 cm.

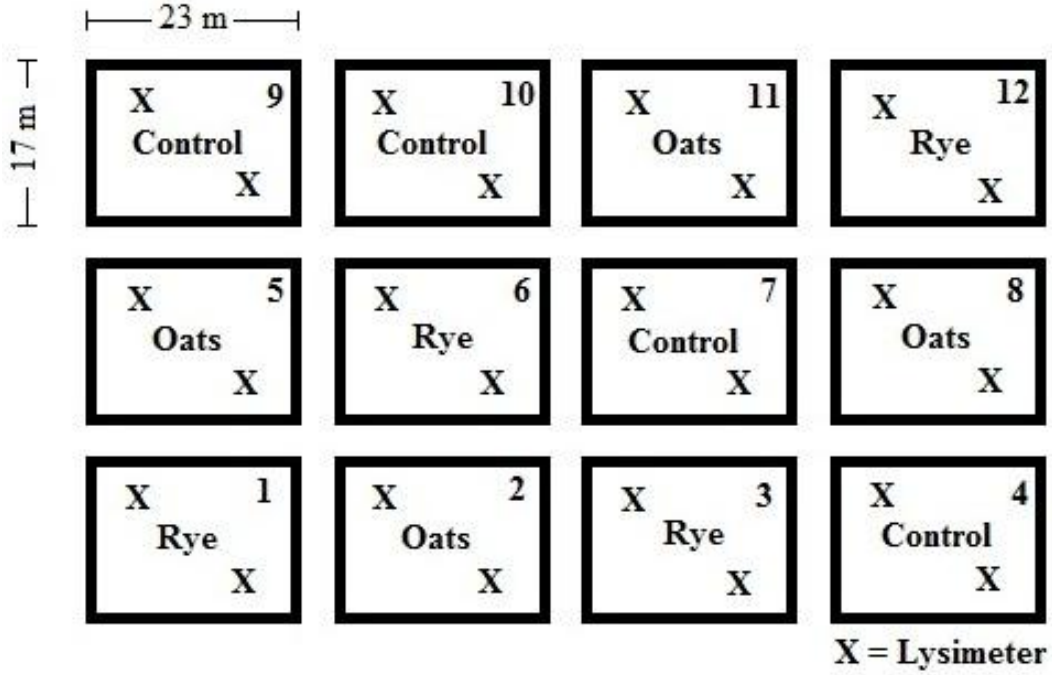


**Figure 1**. Spatially-balanced complete block plot layout. Plots show both cover crop treatment and the placement of two lysimeters per plot (X).

### *2.3.3 Soil and Water Sampling*

Soil samples were taken periodically throughout the spring season. On day of year 2011 (DOY) 77, 97, 118 and 146 soil was sampled from the 0-to-15 and 15-to-30 cm soil layers for mineral N analysis. Additional cores were taken for bulk density and $N_2O$ analysis (DOY 97, 123 and 144). Tension lysimeters (13.6 cm h x 5.2 cm ID; Soil Moisture Equipment Corp., Santa Barbara, CA) were installed on 24 September 2010 at a depth of 55 cm. On DOY 97, 118 and 146, a water sample was obtained after applying a 70-80 kPa tension through a battery-operated pump, maintained for 24 hours. The pore water was then collected and analyzed for $NO_3$ concentration.

### *2.3.4 Soil and Water Analysis*

Soils collected from the field were placed in a drying oven at 60°C for 24 hours and then sieved through a 2 mm mesh screen. The procedure to determine ammonium and nitrate

concentrations was based on that of Keeney and Nelson (1982) with minor modifications. An 8-gram subsample was extracted from both soil layers with 40 ml of 2M KCl from each replication and mixed on a reciprocal shaker for 30 minutes, then filtered through ashless filter paper (Whatman no. 42). Ammonium and nitrate concentrations from soil extracts and water samples were determined using a segmented-flow autoanalyzer (Seal Analytical, Mequon, WI).

Additional samples from the 0-to-15 cm soil layer were taken for determining potentially mineralizable N (PMN) and active carbon. PMN measures a soil's capacity to mineralize organic nitrogen. Soil materials were prepared in the same manner as previously described for $NH_4$ and $NO_3$ analysis, based on an anaerobic incubation method described by Drinkwater et al. (1996). Briefly, $NH_4$ from paired soil samples is extracted before and after a 7-day incubation period at 37°C. The first sample serves as the initial $NH_4$ present, which is then subtracted from the final $NH_4$ concentration at t = 7 days for the total mineralized N. Ten ml of DDI water was added to the sample and the container was purged with $N_2$ to ensure anoxic conditions during incubation. The method of extraction for $NH_4$ in the final sample was the same as the initial sample, however, 2.67 M KCl was substituted for 2M KCl.

Biologically active carbon was determined according to Weil et al. (2003). Briefly, a 2.5 g soil sample was reacted with 20 ml of .02 M potassium permanganate ($KMnO_4$) solution over a two-minute shaking period. The solution was centrifuged and the resultant supernatant was measured for absorbance at 550 nm using a hand-held colorimeter (Hach, Loveland, CO).

### *2.3.5 $N_2O$ Procedures and Analysis*

Three undisturbed soil cores, 13.6 cm h x 5.2 cm ID, were taken from each plot using PVC pipe for a total of 12 cores per treatment. For destructive analysis, 9 additional cores were collected for sampling during the incubation period. The cores were placed in an incubator

simulating the average air temperature for that time of year (10°C for DOY 97, 15°C for DOY 123 and 20°C for DOY 144) for 24 hours prior to the first sampling (t=0). At this time, the headspace from the sealed cores was sampled followed by a 5-minute simulated rainfall of 150 mm $hr^{-1}$ (12.5 mm total) using a drip-type rainfall simulator (Ogden et al., 1997). Additional samples were taken at hour 6, 12, 24, 48, 72 and 96.

For each sampling, three blanks and all cores were placed in 1-L Mason jars fitted with an airtight seal for one hour (Millar and Baggs, 2004). Because the gas flux was found to be linear over this time period, flux can be calculated as the difference between the sample and the blank over the 1-hour period. A 20 ml sample was taken from the headspace of each jar with a syringe and injected for storage in 12 ml evacuated glass vials (Labco, Wycombe, UK). All samples were analyzed within ten days using a Shimadzu GC-14A gas chromatograph fitted with an electron capture detector operating at 325°C (Shimadzu, Tokyo, Japan). Denitrification rates were calculated using a formula similar to Hernandez-Ramirez et al. (2009) where:

$$N_2O \text{ production rate} = \beta(V_{hs} + V_{lp}\alpha) / S \quad [1]$$

and $\beta$ is the measured $N_2O$ production rate ($\mu g\ L^{-1}$), $V_{hs}$ is the headspace volume (L), $V_{lp}$ is the liquid phase (L), $\alpha$ is the Bunsen absorption coefficient in water (Tiedje, 1982; Christensen and Tiedje, 1988), and S is the incubated soil mass (kg).

### *2.3.6 Statistical Analysis*

Statistical analysis was done using a combination of SAS (PROC MIXED) and R statistical software programs. Nitrous oxide emissions were analyzed using a mixed model to test both main effects and interactions over time using log-transformed data. Results were subsequently back-transformed for illustrative purposes with averages presented as geometric means. With one exception, all other data were tested for normality and heteroscedasticity and

analyzed using two-way ANOVA. Nitrate leachate was found to be severely negatively skewed, which did not allow for transformation. Welch's t-test was used to analyze treatment effects on these data.

## 2.4 Results and Discussion

### *2.4.1 Cover Crop Biomass Accumulation and N Contents*

The rye cover crop produced much higher levels of biomass than the oat cover crop during the fall season (Table 1). Aboveground biomass was nearly 300 kg $ha^{-1}$ greater in the rye plots. In total, rye produced 3.02 times more biomass. Similarly, rye N uptake was 16.27 kg $ha^{-1}$ or 269% greater than the oat cover crop. Additionally, spring biomass production and N-uptake by the rye crop likely played a large role in N cycling dynamics. In total, biomass production averaged nearly 6.95 Mg $ha^{-1}$. N-uptake by the total plant averaged 76.61 kg $ha^{-1}$, with roughly 68 kg $ha^{-1}$ in the aboveground biomass (Table 1).

**Table 1.** Biomass accumulation, C:N and N-uptake for the three harvest sample dates. Different letters within a column represent significant differences at $\alpha = 0.05$.

| | *3 Dec 2010* | | |
|---|---|---|---|
| | Total Biomass (kg $ha^{-1}$) | C:N | N-Uptake (kg $ha^{-1}$) |
| *Oat Shoots* | 156.04 (16.34)a | 7.67 (0.04)a | 8.62 (0.88)a |
| *Oat Roots* | 37.93 (6.64)b | 14.06(0.68)b | 1.00 (0.23)b |
| *Rye Shoots* | 455.3 (46.65)c | 8.91 (0.24)c | 22.8 (2.52)c |
| *Rye Roots* | 130.04 (17.2)a | 18.05 (1.38)d | 3.09 (0.61)d |
| | *28 April 2011* | | |
| | Total Biomass (kg $ha^{-1}$) | C:N | N-Uptake (kg $ha^{-1}$) |

| | | | |
|---|---|---|---|
| *Oat Shoots* | - | - | - |
| *Oat Roots* | - | - | - |
| *Rye Shoots* | 1208.19 (164.68)a | 15.43 (0.56)a | 24.29 (4.70)a |
| *Rye Roots* | 307.32 (39.23)b | 24.53(1.08)b | 3.54 (0.32)b |
| | *26 May 2011* | | |
| | Total Biomass ($kg\ ha^{-1}$) | C:N | N-Uptake ($kg\ ha^{-1}$) |
| *Oat Shoots* | - | - | - |
| *Oat Roots* | - | - | - |
| *Rye Shoots* | 5926.37 (800.10)a | 27.43(4.71)a | 67.52 (11.07)a |
| *Rye Roots* | 1020.30 (167.29)b | 32.43(1.33)a | 9.09 (0.97)b |

### *2.4.2 Nitrate Leaching*

Treatment effects on nitrate leaching concentrations out of the root zone (55 cm.) were found to vary considerably (Fig. 2). Rye significantly and markedly decreased $NO_3$-N concentrations over the control and oat treatments. Concentrations among the other two treatments remained high throughout the spring season with an increasing trend through the early part of the spring season and then a gradual decrease with the oats generally maintaining higher average concentrations throughout the sample period. Throughout the spring season, average measured nitrate levels were 43.36, 51.96 and 0.82 mg $NO_3$-N $L^{-1}$ for the control, oat and rye plots, respectively.

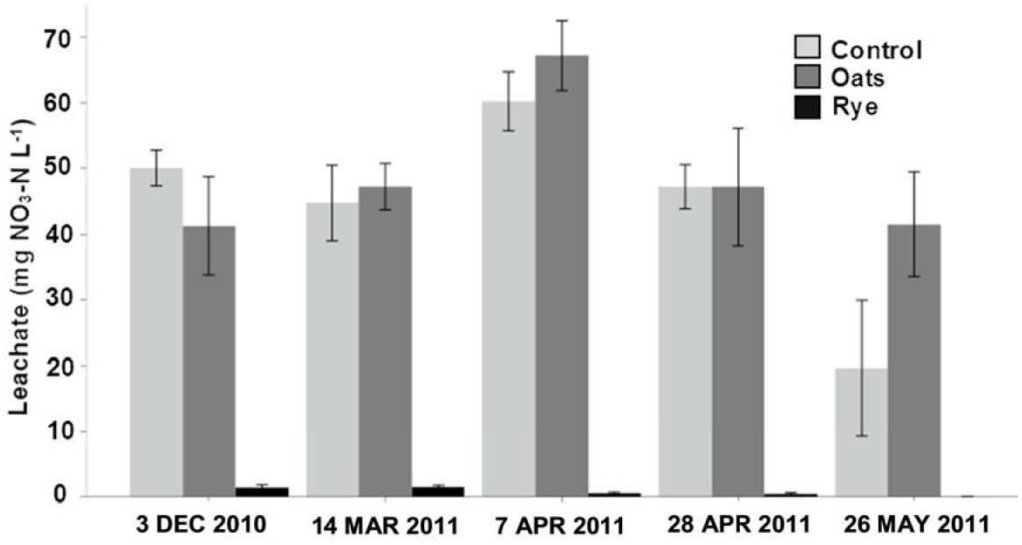


**Figure 2.** $NO_3$-N concentrations measured from lysimeters within each plot throughout the spring season. Lysimeter depth was 55 cm, which was found to be below the cover crop root zone. Due to field dryness, the rye sample during the late spring period is an average of two lysimeters.

### *2.4.3 $N_2O$ Emissions*

While variability was high, both spatially and temporally, significant results were found in nitrous oxide emissions. Clear trends emerged particularly when replications are averaged over treatments. Treatment effects changed as the spring season progressed (Table 2). The oat treatment produced similar results to the control throughout the sample period while rye decreased $N_2O$ emissions after a high initial flux. This pattern is similar to that of Christenson and Christenson (1991) who noted that initially high spring $N_2O$ flux is likely the result of newly liberated carbon whereas later emissions are sustained by organic matter C.

The present study showed higher emissions at the early sample coming from plots with cover crops, thus providing a relatively fresh carbon source. Presumably emissions later in the season were then driven by the larger organic matter pool, which is confirmed by similar emissions in the oat and control plots. Reductions in the rye plots, however, could have been a result of nitrate pool reductions even though sufficient carbon remained. Average emissions from

the rye treatment were roughly half of the control and oat treatments during the final two samplings. Emissions in the rye were reduced by 57% and 49% over the oat plots and 71% and 46% over the control plots during the final two samplings respectively.

**Table 2.** Total $N_2O$ emissions (μg $N_2O$-N $kg^{-1}$) over the entire sample period (96 hours). Values in parentheses represent ± one standard error of the mean. Different superscripts within a column represent significant difference at $\alpha = 0.05$. Because of the lognormal distribution of emissions data, averages are presented as geometric means.

| | Average Cumulative Emissions (μg $N_2O$ -N kg-$soil^{-1}$) | | | |
|---|---|---|---|---|
| | *7 April 2011* | *28 April 2011* | *26 May 2011* | *Geometric Average* |
| *Control* | 33.46 (4.84)a | 73.74 (7.74)a | 51.91 (11.74)a | 50.41 |
| *Oat* | 56.07 (11.46)a | 49.20 (11.32)a | 54.46 (10.01)ab | 59.55 |
| *Rye* | 73.74 (7.46)a | 21.38 (1.93)b | 28.01 (0.97)b | 35.35 |
| Geometric Average | 51.72 | 47.48 | 42.94 | |

The majority of emissions occurred during the first 48-72 hours of each incubation (Fig. 3). Particularly between the oats and control treatments, variability was extremely high with an average CV of 55% and 67% of the mean respectively. In contrast, emissions for the rye treatment were far more stable and varied by less than half of that of the other treatments.

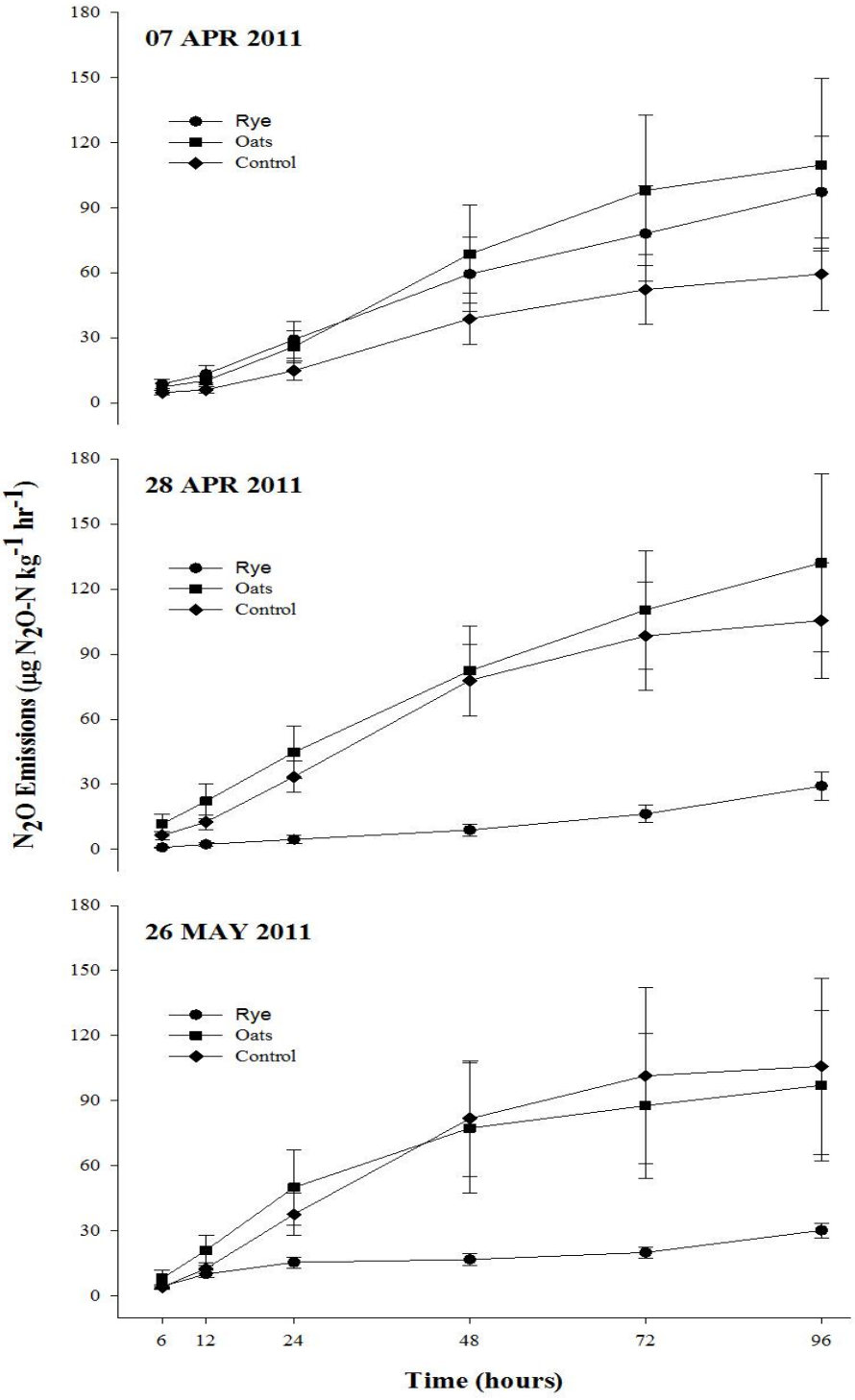


**Figure 3.** 96-hour cumulative $N_2O$ emissions at different times during the early growing season. Means represents the geometric average for each treatment and error bars equal ± one SEM.

### *2.4.4 Soil Characteristics and Mechanisms for $N_2O$ Emissions*

$N_2O$ production in this experiment represents a simulated heavy-rainfall event during the spring season, and the trends offer insight into the dynamics of $N_2O$ production during this volatile period with the onset of a heavy rainfall. Compared to a control-fallow treatment, both rye and oats produced much more $N_2O$ on average during the early spring yet, statistically significant differences were not detected. As the season progressed, however, $N_2O$ emissions decreased significantly in the rye treatment while emissions from both the control and oat treatments increased.

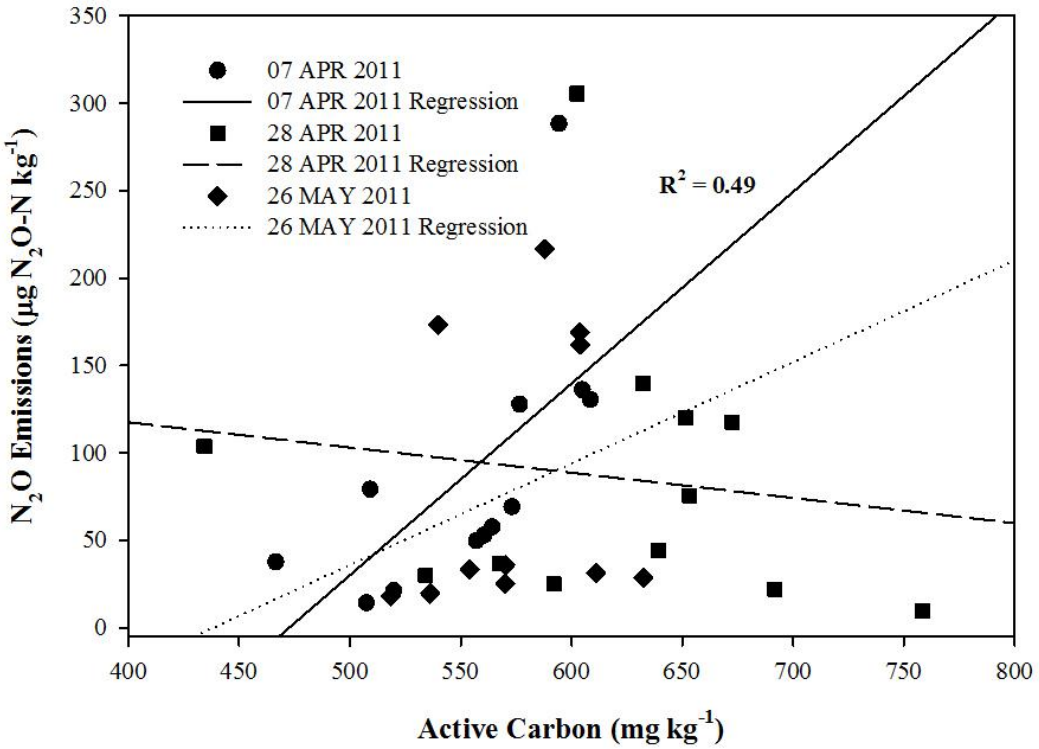


**Figure 4.** Linear regression of $N_2O$ emissions on active carbon. Active carbon was a significant predictor only during the early spring period (7 April 2011).

Wagner-Riddle et al. (2008) demonstrated that high $N_2O$ production during spring-thaw events was a result of newly liberated carbon and available nitrate through freeze-thaw processes

in surface soil layers. Based on linear regressions, active carbon was positively correlated ($p = 0.004$) with nitrous oxide production during the early spring sample period. However, active carbon did not correlate well with emissions later in the season (Fig. 4). Furthermore, nitrate, sampled from the top 30 cm was found to be a significant predictor variable only in the mid-spring sample (Fig. 5). These results, combined with the trends shown in Table 2, suggest that throughout the spring season, $N_2O$ emissions rely on a complex interaction between variables with relative importance shifting depending on the sample date.

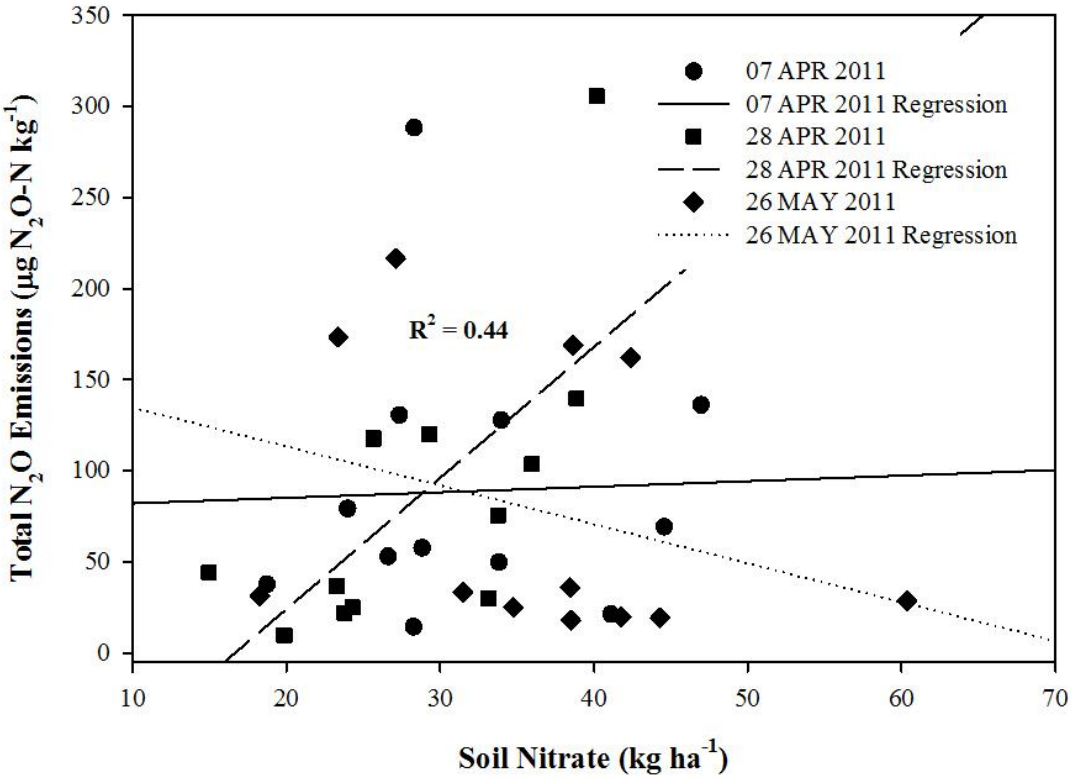


**Figure 5.** Linear regression of log emissions on soil nitrate levels in the top 30 cm. Nitrate was a significant predictor ($R^2 = 0.44$) only during the mid-spring period (28 April 2011)

Additionally, nitrate appears to be the primary limiting factor in $N_2O$ production. Within the rye plots, soil $NO_3$ contents were significantly reduced during the mid-spring period, which corresponds to the period of high N-uptake by the rye crop (Table 1). $N_2O$ emissions were

reduced correspondingly (Fig. 3). In the late spring, however, $NO_3$ rebounded in the rye plots with higher levels of active C, yet $N_2O$ production remained lower on average. While the latter differences were statistically significant between the rye and control plots, the lower emissions are difficult to interpret based on these measures and perhaps indicate that physical differences affecting oxygen diffusivity or the microbial population should be examined further to help explain these results.

The emissions of the mid-Spring period corroborate work from previous lab studies. Parkin et al. (2006) found that a rye cover crop reduced $N_2O$ emissions after the addition of swine manure, particularly at high rates of application. Similarly, McSwiney et al. (2010) noted that $N_2O$ production was decreased in maize plots following winter rye cover when fertilization rates were applied in excess of crop physiological need. The effect in the latter is likely a result of N immobilization after rye incorporation. In concert, rye could potentially reduce emissions during its growth phase and into the primary growing season when used as a green manure. In field studies, however, this effect has not been conclusively established (Parkin and Kaspar, 2006).

In the rye plots, N-uptake regulates the nitrate pool size, a proximal driver of $N_2O$, by outcompeting bacteria for available nutrients (Smith and Tiedje, 1979). Another indirect or distal factor controlling emissions among the treatments is the combined effects of mineralization and immobilization. Parkin et al. (2006) speculated that recent manure additions may in fact increase mineralization, which could lead to increased $N_2O$ emissions. Biomass incorporation stimulates these processes and its effect on $N_2O$ emissions has been an area of study.

In a rice-wheat cropping system, Aulakh et al. (2001) found that initial $N_2O$ emissions after rice transplanting were higher in plots receiving a narrow C:N green manure over plots

amended with a higher C:N ratio wheat residue. Over a longer time scale, however, the trend was reversed, which the authors theorized was the result of mineralization and long-term availability of carbon. This could explain the sustained emissions in the oat plots in this study, which remained higher throughout the entire spring period with soil nitrate levels remaining fairly stable (Table 3). At the time of winter-kill, the oat plots had accumulated an average of 8.62 kg $ha^{-1}$ of N in the aboveground biomass and another 1 kg $ha^{-1}$ of N in root biomass. Oats die with the onset of frost and the biomass is returned to the soil. Additionally, the coefficient of variation ($\sigma/\mu$) in emissions from oat plots varied much more as the season progressed. Dispersion increased from approximately 15% in the early spring to 32% in the late spring.

**Table 3.** Soil nitrate and active carbon levels in the top 30 cm at the three sample periods. Numbers in parentheses are the standard errors of the mean. Statistical differences were not detected.

| *Treatment* | *7 April 2011* | | | | |
|---|---|---|---|---|---|
| | $NH_4$-N (kg $ha^{-1}$) | | $NO_3$-N (kg $ha^{-1}$) | | Active C (mg $kg^{-1}$) |
| | 0-15 cm | 15-30 cm | 0-15 cm | 15-30 cm | |
| Control | 7.54 (0.66) | 4.76 (0.89) | 33.06 (3.77) | 34.74 (4.14) | 528.59 (25.61) |
| Oats | 4.16 (0.98) | 7.23 (1.19) | 32.64 (3.97) | 40.58 (3.78) | 545.01 (19.37) |
| Rye | 5.58 (01.36) | 5.45 (1.18) | 33.63 (5.45) | 35.7 (4.94) | 586.73 (11.83) |
| | *28 April 2011* | | | | |
| | $NH_4$-N (kg $ha^{-1}$) | | $NO_3$-N (kg $ha^{-1}$) | | Active C (mg $kg^{-1}$) |
| | 0-15 cm | 15-30 cm | 0-15 cm | 15-30 cm | |
| Control | 42.36 (7.30) | 40.13 (7.60) | 31.76 (2.71) | 30.6 (5.48) | 648.9 (8.84) |
| Oats | 52.48 (8.82) | 43.53 (9.23) | 30.61 (4.14) | 36.44 (4.01) | 570.47 (47.36) |
| Rye | 57.63 (9.03) | 42.74 (9.51) | 22.3 (2.85) | 23.91 (2.79) | 637.95 (52.59) |

| | *26 May 2011* | | | | |
|---|---|---|---|---|---|
| | $NH_4$-N (kg $ha^{-1}$) | | $NO_3$-N (kg $ha^{-1}$) | | Active C (mg $kg^{-1}$) |
| | 0-15 cm | 15-30 cm | 0-15 cm | 15-30 cm | |
| Control | 55.73 (10.01) | 50.97 (9.83) | 36.51 (13.49) | 45.36 (12.05) | 561.63 (20.41) |
| Oats | 58.27 (7.98) | 36.8 (7.97) | 35.39 (10.50) | 30.38 (11.47) | 558.51 (15.63) |
| Rye | 47.41 (4.99) | 48.1 (4.04) | 37.95 (8.66) | 40.54 (10.53) | 595.94 (15.55) |

The increasing coefficient of variation and sustainment of higher average emissions was possibly the result of increased rates of oat residue decomposition as temperatures increased throughout the spring thus mobilizing N. High nitrate levels in lysimeters beneath oat plots toward the end of the spring season corroborate this hypothesis, particularly in comparison with fallow plot leachate.

Increased decomposition may also explain the high variability and occurrence of "hotspots" within these plots (Parkin, 1987, McClain et al., 2003). Heterogeneous decomposition patterns would likely produce localized areas of relatively high $NO_3$ and/or labile carbon contents. Denitrification variability decreased within the rye plots later in the spring season (Fig. 3), which, perhaps due to more uniform coverage, resulted in labile nitrate being consumed in a more homogenous fashion and decreasing the possibility for localized denitrification zones. Additionally, PMN did not vary significantly among treatments and mineralization potential remained fairly stable throughout the sample period (Fig. 6), which supports the notion that deposited carbon and nitrate from the oat biomass increased the likelihood of denitrification microsites due to fresh biomass additions as opposed to more complicated microbial interactions (mineralization/nitrification) among treatments. Heavy tillage, manure inputs and living plants

can increase mineralization rates (Wander et al. 1994). The oats were not incorporated and that which was cycling through SOM may actually have a neutralizing effect through increased activity by fresh inputs offsetting immobilized nutrients due to its high C:N ratio.

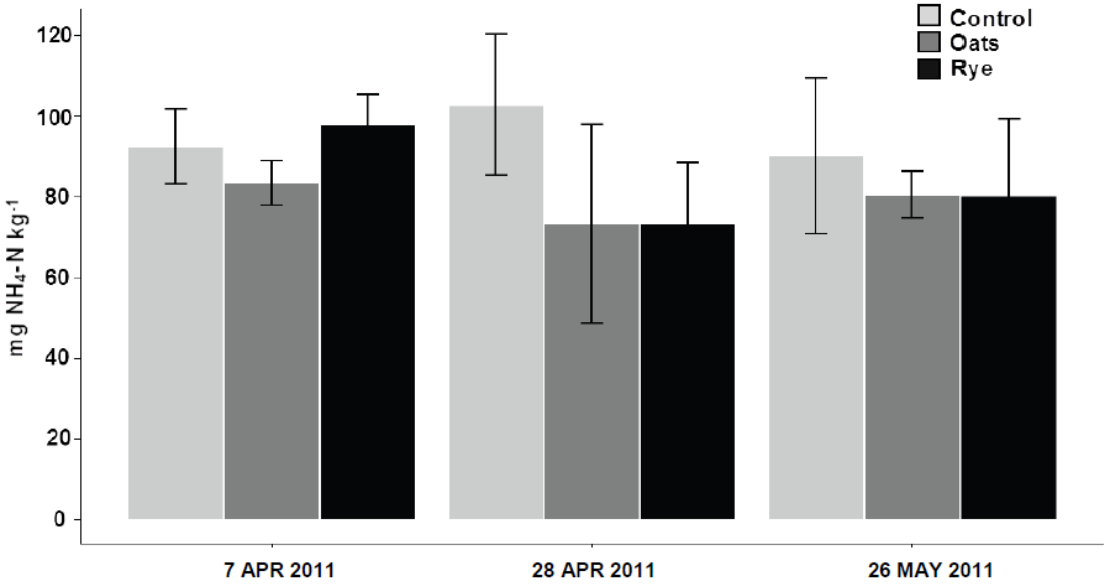


**Figure 6.** Potentially mineralizable nitrogen measured at the 0-15 cm depth during the spring season.

## 2.5 Conclusion

During the volatile spring period when field N losses can be high, rye cover crops show potential to mitigate negative environmental effects. In this treatment, nitrous oxide emissions resulting from a simulated heavy rainfall event were highest in the early spring, averaging nearly 33 g N $ha^{-1}$ $day^{-1}$ with a 70% decrease during later portions of the spring when the rye was actively taking up N and producing biomass. Oats on the other hand averaged consistently higher emissions throughout the spring period with approximately 27 g N $ha^{-1}$ $day^{-1}$. In the control/fallow plots emissions and variability increased as the spring progressed averaging 14 g N $ha^{-1}$ $day^{-1}$ and increasing to nearly 28 g N $ha^{-1}$ $day^{-1}$ for the remainder of the sampling.

Rye had an even greater impact on reducing leaching concentrations lower in the soil profile. Nitrate concentrations in the rye plots were extremely low throughout the sampling period and

decreased during the spring. Oats, when compared to the control treatment, showed no improvements in decreasing nitrate concentrations at 55 cm. Further nitrous oxide losses may be expected from leached nitrates at the terrestrial-aquatic interface (Clough et al., 2007), thereby amplifying the treatment effects.

This trial investigated nitrate losses under winter cover crops from fields with a history of manure management during the spring season. To remove the effects of seasonal climatic variation, nitrous oxide emissions were measured by simulating a heavy rainfall at the average ambient temperature at a given sample date. Due to the inherent physiological differences, notably its ability to survive cold temperatures, rye had a much more significant and positive effect on reducing nitrate leachate concentrations and denitrification losses than oats. Therefore, winter hardy cereal cover crops should be given strong consideration over winter-killed cover crops when environmental N losses are a concern.

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# Chapter 3

## Nitrous oxide emissions and denitrification molar ratios on soils with manure and synthetic fertilizer histories


### 3.1 Abstract

The denitrification process is a significant loss pathway in the nitrogen cycle. Carbon content, often found at elevated levels in manured soils, can play a critical role in regulating nitrous oxide emissions. Nitrate content and oxygen status are the other primary drivers of emissions, yet the interaction of these three variables requires further examination to elucidate the dynamics of the denitrification process. Emissions of $N_2O$ and $N_2$ were measured for 168 hours from repacked soil cores at 80% water-filled pore space after the application of 0, 50, 100 and 200 kg $ha^{-1}$ of labeled-$K^{15}NO_3$ to New York State soils historically amended either with cattle manure (M) or no manure (NM).

Significant differences were found in the $N_2O$ emissions profiles between the two treatments with a simultaneous increasing trend in emissions with increasing fertilizer applications. M soils produced 53-, 15.5- and 8.6-fold increases in $N_2O$ emissions over NM soils. Additionally, the mean ratio of nitrous oxide to total denitrification ($N_2O$: $N_2O + N_2$) was higher for M soil and increased with fertilizer rate at values of .17, .25 and .43 for fertilizer rates of 50, 100, 200 kg $ha^{-1}$. The respective ratios in the NM soil was .01, .03 and .14. In conclusion, $N_2O$ emission potential is of significant concern where the $NO_3$-N pool is in abundance, combined with heavily manured fields where organic carbon content is typically high.


### 3.2 Introduction

Nitrous oxide ($N_2O$) losses from arable lands affect both the individual farmer and society as a whole. To the farmer, losses of nitrogen (N) mean the loss of a principal nutrient in plant

growth, a potential yield deficit, and a reduction in income. To society, N loss through denitrification is costly in several ways: $N_2O$ is extremely efficient as a heat trapping gas, and its global warming potential is 310 times greater than $CO_2$ on a per molecule basis (IPCC, 2007). In addition, $N_2O$ is one of several ozone-depleting substances (Crutzen, 1970) and it is now the most abundant anthropogenic emission source by an estimated factor of ten over all other sources (Ravishankara et al, 2009).

Including $CO_2$ offsets, the US agricultural sector is responsible for emitting an estimated 462 Tg of carbon dioxide equivalents ($CO_2$ eq.; EPA, 2010). Crop production accounted for over 30% of these emissions, with $N_2O$ emissions totaling 154 Tg $CO_2$ eq. (USDA, 2011). A primary driver of $N_2O$ emissions is the added N through synthetic and organic sources of fertilization. $N_2O$ emissions result primarily from two often-coupled soil redox processes, nitrification and denitrification, which are tightly regulated by the availability of carbon and oxygen, as well as the soil redox state. Denitrification is often considered the predominant source of $N_2O$ emissions (e.g., Bateman and Baggs, 2005) and is the focus of this study.

### *3.2.1 Denitrification Process*

The process of denitrification closes the N cycle, returning $N_2$ gas to the atmosphere. This mechanism involves the sequential reduction of $NO_3$ to $N_2$ in the following steps (with the participating enzymes, Nitrate Reductase (Nar), Nitrite Reductase (Nir), Nitric Oxide Reductase (Nor) and Nitrous Oxide Reductase (Nos) listed under each; Tiedje, 1994):

$NO_3$ (ionic oxide) $\rightarrow$ $NO_2$ (ionic oxide) $\rightarrow$ NO (gas) $\rightarrow$ $N_2O$ (gas) $\rightarrow$ $N_2$ (gas)

Nar $\rightarrow$ Nir $\rightarrow$ Nor $\rightarrow$ Nos

This process is primarily mediated by a variety of heterotrophic microbes, which operate to a large extent in oxygen-limited environments. The participating enzymes are not extremely sensitive to $O_2$ concentrations with the exception of Nos. Because of its $O_2$ intolerance, oxygen exposure deactivates the enzyme, the only known to reduce $N_2O$ to $N_2$. This generally causes an upward shift in the N2O:N2 ratio (Richardson et al, 2009).

Along with pH and temperature, the availability of nitrogen oxides and organic carbon are generally thought to be the other main regulators of denitrification and all interact in a complex fashion (Tiedje, 1988). A variety of studies have found a positive correlation between denitrification and the addition of mineral N. Even as oxygen is depleted, there appears to be a baseline level of inorganic N required for significant emissions to occur (Dobbie et al, 1999). Similarly, carbon availability has long been known to play a significant role in denitrification (Burford and Bremner, 1975). Reactive carbon is often supplied in manure additions. Paul and Beauchamp (1989) found that these increased levels particularly that of volatile fatty acids (VFA), correlate with increased denitrification in waterlogged soils.

VFAs generally degrade within a few days. Thus, it might be expected that additions of manure, high in VFAs, will stimulate gas emissions. Stevens and Laughlin (2002) found that concurrent application of cattle slurry and inorganic fertilizer significantly increased overall emissions while application of inorganic fertilizer 3 to 4 days after the application of cattle slurry had no effect on emissions. Additionally, Stevens and Laughlin (2001) found that cattle slurry increased emissions when applied with synthetic fertilizer by an average of 0.63% of applied N.

Moreover, recent literature suggests that nitrous oxide emissions increase exponentially with increasing N application (Hoben et al, 2010; Ma et al, 2009; Zebarth et al, 2008; McSwiney and Robertson, 2005). However, very little has been discussed on this phenomenon with respect

to increased levels of active carbon as one might expect in manured soils. Specifically, how does the addition of manure interact with increasing N additions with respect to the products of denitrification? Accordingly, the objectives of the current study were to examine the effect of a history of manure inputs on both the denitrification process and the molar ratio or partition of $N_2$:$N_2O$ as a result of N fertilizer application.

Figure 1 provides a clear representation of the factors that control the molar ratio of $N_2$:$N_2O$ during denitrification (Richardson et al., 2009). While this is a generalization, excluding myriad interactive effects, we hypothesize that carbon will drive total denitrification to a greater extent in the manured soils, which is assumed to be in greater supply over the non-manured soil. Moreover, the molar ratio should increase in favor of $N_2O$ as $NO_3$ increases by outcompeting $N_2O$ as the terminal electron acceptor and/or inhibiting reduction to $N_2$ (Blackmer and Bremner, 1978, Gillam et al., 2008). Thus, the objectives of this study were to examine the denitrification potential in traditionally manured and non-manured soils while also testing the variation in the molar ratio of $N_2$:$N_2O$ with increasing fertilizer rates.

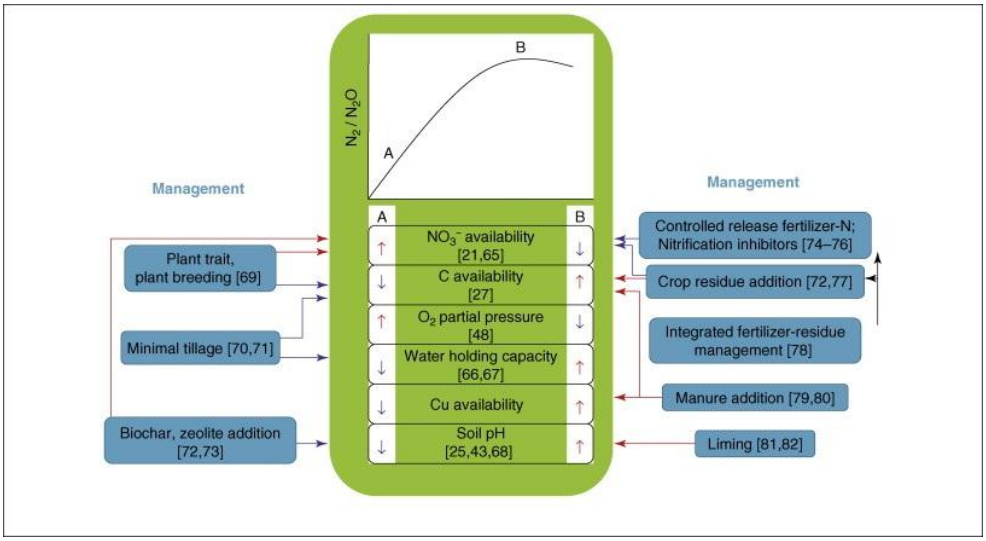


**Figure 1.** Factors controlling the ratio of $N_2$:$N_2O$ produced during denitrification. Adapted from Richardson et al. (2009).

## 3.3 Materials and Methods

### *3.3.1 Core Preparation and Incubation*

The experiment was conducted using silt-loam soils from two fields with a traditional corn (*Zea mays* L.)-based cropping rotation. The soils were chosen based on their similarity in texture, both being dominant in the silt fraction. The first field (M) was a working dairy farm with a history of manure application located in Lansing, NY, USA (42°35' N, 76°31'W). The soil at this site is an Ovid silt-loam (fine-loamy, mixed, active, mesic Aeric Endoaqualf). During the past three years, manure was applied on 10 April 2008, 5 October 2009 and 15 April 2010 (final application before study commencement) at rates of 64, 75 and 43 $m^3$ $ha^{-1}$, respectively. The average N content for liquid manure on the farm is approximately 2.5 kg $m^{-3}$.

The second site (NM) was a non-manured soil with a corn-based rotation classified as a Honeoye silt-loam (fine-loamy, mixed, mesic Glossoboric Hapludalf) located at the Cornell University research farm near Aurora, NY (46°26' N, 76°26' W). The M soil had slightly higher silt content (55% vs. 50%) while the NM soil had slightly higher clay content (38% vs. 33%). The primary difference is organic matter content with 4.1% in the M soil and 3.2% in the NM soil in the surface layer. The chemical and textural makeup of the soils is shown in Table 1.

**Table 1.** Physical and chemical characteristics of soils evaluated in the study.

| Treatment | Sand | Silt | Clay | pH | Organic Matter |
|---|---|---|---|---|---|
| | g $kg^{-1}$ soil | | | | % |
| Manure | 330 | 550 | 120 | 7.1 | 4.1 |
| Non-Manure | 380 | 500 | 120 | 7.9 | 3.2 |

Soils were sampled in bulk from the top 15 cm of the soil surface using a tile-spade one week prior to the start of the incubation. Soils were homogenized and stored at 4°C until

commencement of the incubation. To maintain some structural integrity and remove excess debris and rocks, all soil material was sieved using an 8-mm mesh screen and repacked into PVC cores (5.2 cm i.d. x 13.6 cm h) to a bulk density of 1.15, which was representative of field values. The cores were incubated in the dark at 30°C and $N_2O$ and $N_2$ losses were monitored for a period of 168 hours. To induce oxygen deficits and force the denitrification process, all cores were maintained at a water-filled pore space of approximately 80% throughout the duration of the experiment, which was corrected gravimetrically at each sample period.

### *3.3.2 $^{15}$N-Flux Methodology*

Isotopically-enriched $K^{15}NO_3$ (60 atom%; Sigma Aldrich, MO, USA) was added in solution in a factorial arrangement to the manured and non-manured soils at 0, 50, 100 and 200 kg $ha^{-1}$ with 4 replications for a total of 32 cores in a manner using similar to Panek et al. (2000). Briefly, all cores were wetted to 9 ml below the desired WFPS using DDI water. $K^{15}NO_3$ was dissolved in solution at desired rates and injected in 3 aliquots of 3 ml in different locations of the core by syringe. To achieve a uniform distribution throughout the core, the syringe was withdrawn slowly from the soil while injecting each aliquot.

Gas samples were taken on 6 separate occasions beginning at 12 hours after fertilizer injection and thereafter at 24, 36, 48, 120 and 168 hours. Sampling was conducted similar to previous studies (Millar and Baggs, 2004; Gentile et al., 2008) in which a one-hour flux is measured after cores were placed in 1-L Mason jars fitted with an airtight seal and sampling septum. Three empty jars were incubated to serve as reference (blank) samples. Because the gas flux was found to be linear over this time period, flux can be calculated as the difference between the sample and the blank over the 1-hour period. A 15 ml sample was taken from the headspace of each jar with a syringe and injected for storage in 12 ml evacuated glass vials (Labco;

Wycombe, UK).

All samples were processed at the University of California-Davis Stable Isotope Facility for $N_2$ and $N_2O$ concentrations and isotopic signature with a ThermoFinnigan GasBench + PreCon trace gas concentration system interfaced to a ThermoScientific Delta V Plus isotope-ratio mass spectrometer (Bremen, Germany). After isolation and concentration of gases, each sample is transferred by a helium carrier stream; first analyzing $N_2$ gas, which is passed to the IRMS through a molecular sieve 5A GC column (15m x 0.53mm ID, 25°C, 3 mL/min). Simultaneously, the rest of the gas is passed through a $CO_2$ scrubber (Ascarite) and $N_2O$ is trapped and concentrated. $N_2O$ is carried by helium to the IRMS via a Poroplot Q column (25 m x 0.53 mm, 25°C, 1.8 mL/min), which separates $N_2O$ from residual $CO_2$.

The ion currents at *m/z* 44, 45 and 46 for $N_2O$ and 28, 29 and 30 for $N_2$ allow for back-calculation of the enrichment of the denitrifying pool ($^{15}Xn$), assuming uniform mixing of added $NO_3$ with indigenous $NO_3$. Along with the molecular ratios for $N_2O$ and $N_2$ ($^{44}R$, $^{45}R$, $^{29}R$ and $^{30}R$, respectively) in the enriched atmospheres, the fraction proportional to both gases evolved ($N_2/N_2O$) can be calculated (Mulvaney and Boast, 1986; Arah, 1997).

### *3.3.3 Statistical Analyses*

Due to the highly skewed nature of denitrification data, all statistical analyses were performed on log-transformed data to meet the normality assumptions of the statistical tests used herein. Analysis of variance was used to compare treatment means across time periods and simple, linear regression was used to quantify correlation between fertilization rate and emissions. Means separation was found using Tukey's Honestly Significant Difference and significance was determined at $\alpha$=0.05 unless otherwise stated. All statistical computing was done using the R Software package.

## 3.4 Results and Discussion

### *3.4.1 Trends and Variability in Emissions*

Cumulative $N_2O$ emissions were significantly higher in the traditionally manured soil material than in the non-manured soil material (Table 2). As the fertilizer rate increased, M soils produced 53-, 15.5- and 8.6-fold increases in $N_2O$ emissions over NM soils at 50,100 and 200 kg $ha^{-1}$, respectively. Corresponding increases in average emissions of $N_2$ were 1.7, 1.7 and 1.8-fold, although these differences were not statistically significant (Table 2). Average variability between treatments was higher in M soils for $N_2$ emissions (CVs of 55% and 45%, respectively) with the opposite in $N_2O$ emissions with an average CV of 64% in M soils and 93% in NM soils.

**Table 2.** Means (geometric) and ANOVA statistics including Tukey's HSD for treatment effects on $N_2O$ (first table) and $N_2$ (second table) emissions. Significance is determined at $p < .1$ due to high variability. Additionally, comparisons are included as differences in fertilizer rate within management practices (intra-management; M:M and NM:NM) and between management practices (inter-management (M:NM) where factor levels with corresponding letters are not significantly different.

| Management | Fertilizer Rate | Mean **$N_2O$** Emission † | Tukey HSD | | 95% CI | |
|---|---|---|---|---|---|---|
| | kg $ha^{-1}$ | µg kg-$soil^{-1}$ | Intra-management $p < .1$ | Inter-management $p < .1$ | Upper Bound | Lower Bound |
| M | 50 | 539.45 | a | a | 1658.81 | 175.43 |
| M | 100 | 1345.45 | ab | b | 2882.51 | 628.01 |
| M | 200 | 2359.95 | b | c | 5995.13 | 928.98 |
| NM | 50 | 10.18 | c | d | 39.53 | 2.62 |
| NM | 100 | 87.00 | d | e | 328.82 | 23.02 |
| NM | 200 | 273.68 | d | a | 1110.35 | 67.46 |

| Management | Fertilizer Rate | Mean $N_2$ Emission † | Tukey HSD | | 95% CI | |
|---|---|---|---|---|---|---|
| | kg $ha^{-1}$ | μg kg-$soil^{-1}$ | Intra-management $p < .1$ | Inter-management $p < .1$ | Upper Bound | Lower Bound |
| M | 50 | 2561.78 | a | a | 9068.25 | 723.70 |
| M | 100 | 4053.81 | a | a | 6466.59 | 2541.28 |
| M | 200 | 3084.08 | a | a | 7885.57 | 1206.20 |
| NM | 50 | 1476.67 | b | a | 2972.99 | 733.46 |
| NM | 100 | 2413.03 | b | a | 5368.09 | 1084.69 |
| NM | 200 | 1672.27 | b | a | 3628.05 | 770.79 |

† Geometric Mean

In both M and NM soils, average $N_2$ emissions actually decreased between the mid and high rates of fertilizer application although a statistical change was not detected due to high variability (Fig. 2). Conversely, nitrous oxide emissions not only showed significant increasing trends in the M versus NM effect, but also within-group effects for the fertilizer factor. In M soils, an increase of fertilizer rate from the low (50 kg $ha^{-1}$) to mid (100 kg $ha^{-1}$) and low to high (200 kg $ha^{-1}$) produced average increases of 806 and 1821 μg $N_2O$-N per kg-$soil^{-1}$ (approximately 1.5 and 3.3 kg $N_2O$-N $ha^{-1}$), respectively. The analogous increase in NM soils produced higher emissions of 77 and 264 μg $N_2O$-N per kg-$soil^{-1}$ (approximately 0.1 and 0.5 kg $N_2O$-N $ha^{-1}$; Table 2).

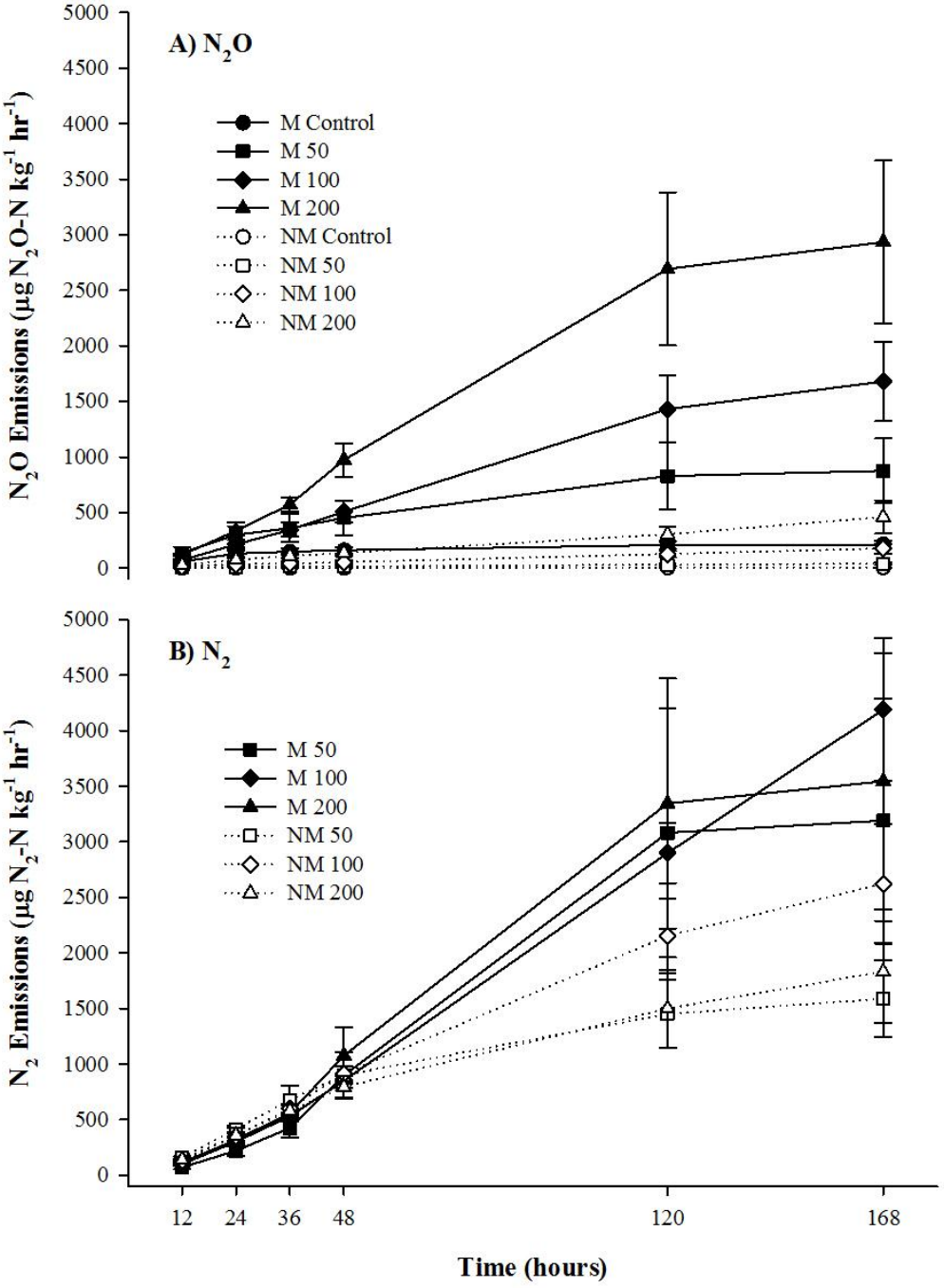


**Figure 2.** Cumulative total emissions of $N_2O$ (A) and labeled $N_2$ (B) from 0 to 168 hours of incubated M and NM soils. Error bars represent ± one SEM. The legend represents manure treatment and N rate.

High variability, particularly for denitrification data, reduces statistical power in the analysis of emissions data. Much of this variation may be attributable to a diffuse collection of active microsite "hotspots" even within re-packed cores. If $O_2$ consumption is relatively high, denitrification microsites may develop irrespective of matrix structure or diffusion rates (Parkin, 1987). Rover et al. (1999) found a CV of 100-to-200% on a relatively small scale - primarily the result of a high number of randomly distributed hotspots across the sample site.

### *3.4.2 Treatment Effects on the Molar Ratio*

An increasing trend with higher fertilizer rate is similarly reflected in the average molar ratio of $N_2O:(N_2O+N_2)$ during the incubation period (Figure 3). The increase in ratio was better represented as a linear trend ($R^2$ = 0.89 and 0.76 for M and NM soils, respectively, Fig. 4) rather than exponential with much higher ratios found in the M soil than in the NM soil overall.

As microsites develop, oxidation of easily available organic carbon triggers $N_2O$ emissions (Parkin, 1987). This may help explain the large $N_2O:(N_2O+N_2)$ ratio differences between the manured and non-manured soils, with organic matter contents of 4.2 and 3.1% respectively.

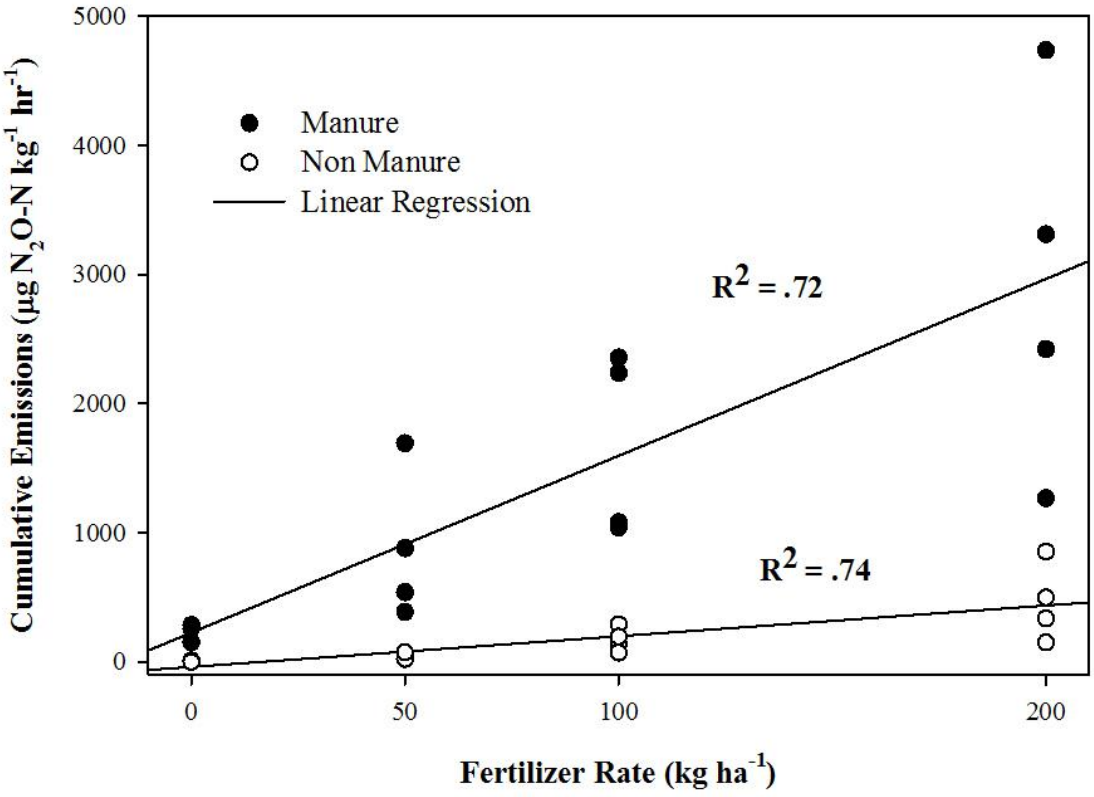


**Figure 3**. Linear regression of cumulative $N_2O$ emissions derived from enriched fertilizer on fertilizer rate for M and NM soils. Fertilizer rate was a significant predictor for $N_2O$ at $\alpha$=0.001. Coefficient of determination ascertained on log-transformed data.

The increased $N_2O$ production in manured soils in this study was similar to those of other researchers. Russow et al. (2008) found that in combination with nitrate fertilizer, soils with higher organic carbon content typically produce higher $N_2O$ rates with the primary production pathway identified as denitrification. Additionally, using soils with a history of manure application, Jagr et al. (2011) found that nitrate additions increased emissions over manure application as soil moisture increased. This effect was reversed at lower moisture. This suggests that at high moisture contents, nitrate was the limiting factor in denitrification.

This hypothesis is supported in the $N_2O$:($N_2O$+$N_2$) molar ratios of the current study.

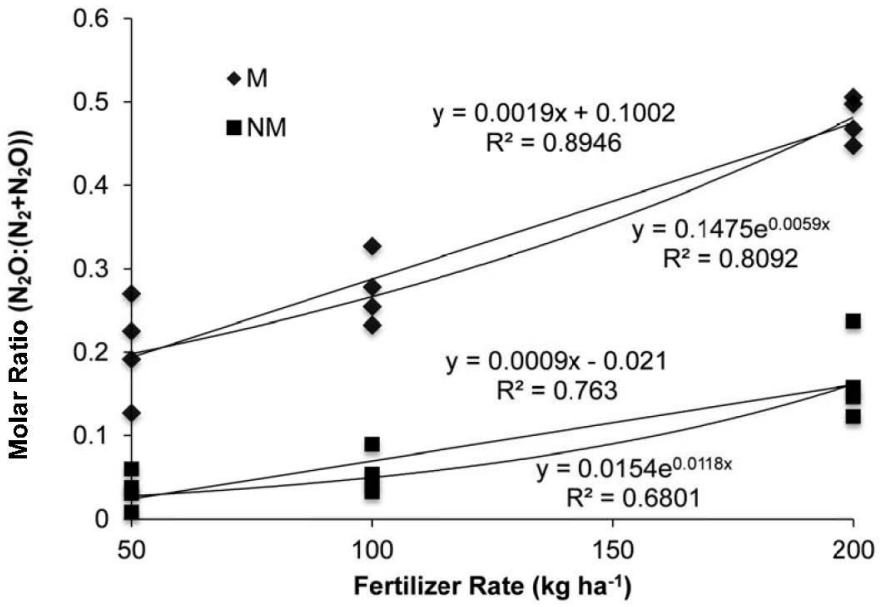


**Figure 4.** Linear and non-linear regressions of the average $N_2O$:($N_2$+$N_2O$) molar ratio as a function of fertilizer rate.

A high $N_2O$:($N_2O$+$N_2$) molar ratio was observed in the M soil at the highest fertilization rate (Fig. 5). With moisture content maintained at 80%, there was very little limitation on $N_2O$ production. This interaction, at higher fertilization rates, highlights the emission potential stimulated through increasing organic carbon pools. Organic matter was 0.9% higher in M soils than in NM soils (Table 1). At high fertilization rates where nitrate was essentially eliminated as a differentiating factor, it is presumed that this carbon source was the primary driver in producing high $N_2O$ emissions.

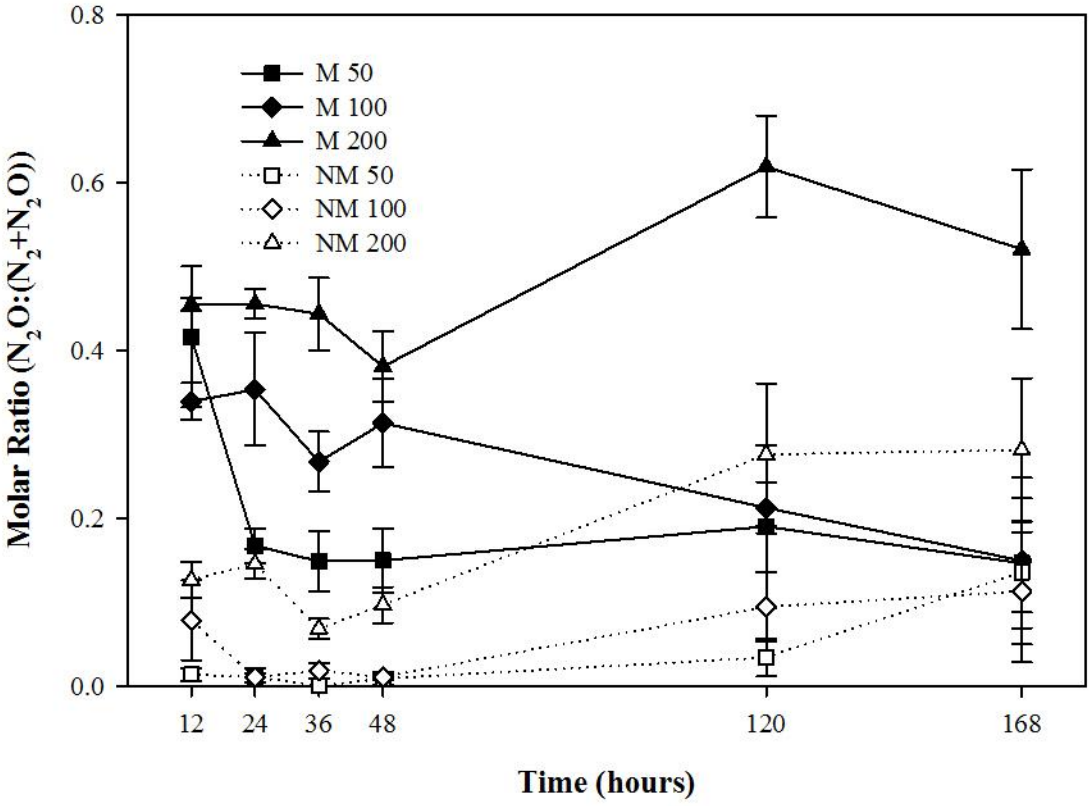


**Figure 5**. Time series of the average molar ratio ($N_2O/N_2 + N_2O$) for M and NM soils. Error bars represent ± one SEM.

In a similar study, Weier et al. (1993) found that at high available C levels, increasing nitrate levels inhibited the conversion of $N_2O$ to $N_2$ with an increase in $CO_2$ levels, which implies a flourishing denitrifying microbial population in which $N_2O$ is the end product. The difference in organic matter in M and NM soils appear relatively minor with respect to the differences in $N_2O$ emissions. Further study should focus on this relationship and the nature of emissions increases with small increases in carbon content.

A rich and diverse microbial population (quality and quantity) itself potentially provides a feedback mechanism for further stimulation of the denitrification process (Cavigelli and Robertson, 2001). In examining differences in microbial communities between soils receiving

manure versus mineral fertilizer, Parham et al. (2003) found differences particularly between the rapidly reproducing r-strategist abundance with high populations in manure-amended soils. The authors postulated that manure inputs provide for proliferation of both r- and K (slower reproducing)-strategists because manure offers a more balanced ratio of inorganic nutrients to organic C. Conversely, inorganic fertilizers (NPK) only supply inorganic nutrients leaving organic carbon as the limiting factor, thus reducing the r-strategist population. Under controlled conditions, Cavigelli and Robertson (2000) found that these differences can have an effect on Nos gene expression, thus shifting the molar ratio toward $N_2$ production with Nos enzymes more active in successional (uncultivated) fields rather than in agricultural fields. A microbial basis for the change in reductase activity suggests that the microbial population existing in the M fields has less capability for $N_2O$ reduction despite the availability of reductant and oxidant. Microbial population was not enumerated in this study, however, and further study is necessary to ascertain how respective populations might have changed between these two fields and how these changes affect emissions factors.

The current yearly emissions factor recommended by the IPCC is 1% of N supplied either through synthetic or organic sources (IPCC, 2006). The $N_2O$ emissions factors for this study were approximately 3% and .3% for M and NM soils respectively. While these values from 168 h core incubations are not commensurate with annual field losses, they highlight the differences in emission potential and the difficulty in deriving a static emissions factor for yearly emissions over all soils as the $N_2O$:($N_2O$+$N_2$) molar ratio and emission rate are highly variable . Looking at interactive effects, Stevens and Laughlin (2002) calculated an emissions factor of 2.9% when adding manure slurry simultaneously with mineral fertilizer.

Additionally, the role that carbon plays in determining this ratio remains unclear,

particularly in sustaining a high ratio throughout the study period. As Fig. 4 shows, the $N_2O$:($N_2O$+$N_2$) ratio increased with increasing $KNO_3$ for both M and NM. At the beginning of the incubation in nearly all factorial combinations, the $N_2O$:($N_2O$+$N_2$) molar ratio tends toward a higher emission of $N_2O$, where the mechanism is assumed to be a lag between the development of Nar and Nos production at the onset of favorable denitrifying conditions (Letey et al., 1980). Using a single N rate and three incremental C rates, Stevens and Laughlin (1998) found the total denitrification to be similar between the mid and high C rates but the mid-rate had a higher $N_2O$ production rate (higher molar ratio), which they attributed to a nitrate limitation in the high C rate. In the M soils particularly, this study corroborates this finding as a higher $N_2O$:($N_2O$+$N_2$) molar ratio was maintained where N was presumably not limiting (200 kg ha-$^1$) during the entire incubation while at lower rates, the molar ratio declines with time and in the lower carbon content of NM (Fig. 5). Initial carbon content can be assumed to be approximately equal within each treatment, which further implies that nitrate content controlled the trend in the $N_2O$:($N_2O$+$N_2$) molar ratio. When a more balanced nitrate pool is present with that of carbon, it is likely that $N_2O$ emissions will increase.

Additionally, Mathieu et al. (2006) found a moderately significant, positive relationship between $CO_2$ and $N_2$ production, concluding that C (with $CO_2$ as a proxy for C availability) promotes conversion to inert $N_2$. It appears that high $N_2O$ losses may be maintained as long as both factors are found in abundance and will tend toward $N_2$ production particularly as $NO_3$ becomes limiting.

This latter study was confounded by the persistence of cores maintaining a high $N_2O$ rate during the incubation. The authors postulated that, again, microorganismal differences exist with variable ability/sensitivity to reduce $N_2O$. In examining the longitudinal $N_2O$:($N_2O$+$N_2$) data

(Fig. 5), anomalies exist with similar implications: At high N rates for both N and NM soils, a high molar ratio was maintained and generally increased with time. This change was a result of decreasing $N_2$ production while $N_2O$ production remained fairly stable. Carbon quality, particularly in the NM soil, may have affected this as hotspots were created by an expanding microbial population and new, water-soluble carbon sources became bioavailable toward the end of the incubation (Boyer and Groffman, 1996), while $NO_3$ levels remained non-limiting.

**3.5 Conclusion**

Based on these results, the manured soil had a much higher propensity for $N_2O$ production than the non-manured field, producing a nearly 25-fold increase in $N_2O$ emissions when averaged across fertilizer application rates. Under increasing fertilizer N rates, $N_2O$ emissions increased by over 800 μg $N_2O$-N per kg-soil$^{-1}$ between 50 kg ha$^{-1}$ and 100 kg ha$^{-1}$ over 1800 μg $N_2O$-N per kg-soil$^{-1}$ between 50 kg ha$^{-1}$ and 200 kg ha$^{-1}$ in the M soil. Increases in the NM soil were 75 and 260 μg $N_2O$-N per kg-soil$^{-1}$.

The IPCC currently suggests a static emissions factor of applied fertilizer N (IPCC, 2006). A non-linear trend would imply that as the N rate increases, the discrepancy between a fixed emissions factor and observed emissions will increase with applied N (Hoben et al., 2010). While a non-linear change point was not determined, further work is necessary outside of the parameters of the present data set. The results in this study suggest that not only does increasing N affect the $N_2O$:($N_2O$+$N_2$) ratio, there is also an interaction between carbon levels as highlighted by the significantly increased molar ratios in the M soil over the NM soil at corresponding N rates. Thus, in the manured soil with higher carbon content, additional $N_2O$ emissions may be expected at equivalent fertilizer rates due to protracted $N_2O$ emissions under oxygen-deficient conditions without necessarily increasing overall denitrification (Figs. 2 and 3).

Under conditions where high soil organic C content is likely, as is often the case for soils in animal-based agricultural systems (Wander et al., 1994), management practices aimed at controlling the nitrate pool (more precise N fertilizer rates, timing and formulations) are likely to produce more rapid and sustained results in lowering $N_2O$ emissions. This is an intervention at the beginning of the denitrification process as opposed to the alternative of forcing a sufficiently low redox potential to complete reduction to gaseous $N_2$. Additionally, there may be an increased probability of $N_2$ production (as opposed to $N_2O$) of the remaining denitrifying $NO_3$ pool when it is the limiting factor. In general, manure-based systems may exacerbate the increase in $N_2O$ emissions with increasing fertilizer rate, making accounting of nitrous oxide and overall emissions in these systems complicated.

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# Chapter 4

## Nitrogen mineralization of *Faidherbia albida* and maize under different soil quality conditions in Kenya

### 4.1 Abstract

Organic amendments potentially offer a high quality and economical alternative to synthetic fertilizer for increased crop yields. Amendment quality is defined by various indicators; among which is organic carbon quality and the ratio of carbon to nitrogen within the amendment. Carbon content plays a fundamental role in the dynamics of nitrogen release as the amendment is degraded. This interaction was examined using two amendments: Faidherbia albida (FA; *Acacia albida*) and maize stover (MA; *Zea mays* L.) and a mixture with $NH_4SO_4$ applied to two soils converted to agriculture from forest in 1900 and 2002 along a Kenyan agricultural chronosequence. Mineralization was examined through a laboratory incubation study consisting of five sampling events after the initial sampling, occurring on days 7, 15, 30, 50 and 75.

Mineralization patterns followed a similar trend in soils from a recent (New) and much older conversion (Old) to agriculture. Average mineralization in FA amended soils was 4.31% greater than the Control in the New soil and 0.75% in the Old soil during mineralization phases. In contrast, the MA treatment immobilized nitrogen for the majority of the study in both soils and to a greater extent in the more fertile soil. This study demonstrates that both inherent soil quality as well as amendment quality affects N mineralization and crop availability. High quality amendments will likely produce higher yield responses in more degraded soils because of inherent N limitations. With more fertile soils, however, asynchrony in application, particularly of low quality amendments, may be detrimental to short-term crop production.

## 4.2 Introduction

Food insecurity arises for a variety of reasons, from economic inefficiencies in infrastructure and distribution to basic biophysical constraints. Many of the food insecure countries in the world suffer from the former issues while in much of sub-Saharan Africa (SSA), the problem is likely more related to the latter (Sanchez, 2002).

Soil fertility generally refers to a base level of nutrients necessary for crop growth. Contributing factors are both inherent and external. Pertaining to the latter, organic and inorganic sources must be utilized effectively for long-term sustainability. Inorganic fertilizer prices in SSA are 2-6 times higher than in more developed markets (Sanchez, 2002). Factors contributing to high fertilizer prices and volatility include small market size, unnecessary product differentiation, high cost in handling and transport, a poor dealer network and high finance costs among others (Morris et al. 2007). As a result, many farmers place a premium on organic sources of nutrients.

Nitrogen is quite often the primary limiting nutrient in many agroecosystems. Its management varies depending on its source, however, as organic and inorganic sources exhibit different release patterns with respect to plant availability. Inorganic forms of nitrogen are considered readily available, while in the majority of systems (see Schimel and Bennett, 2004) organic forms of nitrogen must first be converted to inorganic forms through the process of mineralization. This process itself is complex and controlled by a multifarious set of variables that include organic carbon quality and the C:N ratio (Palm and Sanchez, 1990). Organic inputs can be characterized along a quality gradient with high quality amendments (N concentration greater than 2.5%, lignin content less than 15% and polyphenolic content less than 4%) considered to contribute positively to net mineralization (Palm and Rowland, 1997).

One such high quality amendment is the leguminous *Faidherbia albida* (Del.) A. Chev. (syn. *Acacia albida*: Mimosoideae). Faidherbia albida (FA) is an acacia common throughout much of SSA and found typically on the plains and savannas near the Sahara desert and in riparian areas of eastern and south-west Africa (Radwanski and Wickens, 1967). FA thrives in climates with an annual rainfall ranging from 50 to 1500 mm (Fagg and Barnes, 1990). Phenotypically, FA is unique in that it bears leaves and fruit during the dry season and sheds its leaves with the onset of the rainy season; eliminating competition for nutrients and water with cash crops. This is enabled by its phreatophytic nature with tap-roots penetrating over 7 m. deep while also developing a dense root network throughout the upper soil profile (Roupsard et al., 2009).

The reverse phenology of FA also provides a natural source of fertility through fresh biomass addition at the start of the primary growing season. Saka et al. (1994) found significantly higher maize yields under FA canopies over unamended soil in the same field. Through the course of one year, Phombeya (1999) calculated that FA litter contributes 129 kg $ha^{-1}$ N and 12 kg $ha^{-1}$ P. Additionally, 24-52% of the N in the leaf litter was derived through atmospheric $N_2$ fixation.

A large body of research exists on nutrient release patterns based on quality indicators, yet much less attention has been paid to the mineralization/immobilization patterns as affected by soils of varying inherent fertility. For example, how does management and age of agricultural land conversion (i.e., soil quality) affect decomposition and nutrient release from the same amendment?

As time is a primary factor in soil development, a chronosequence is essentially a temporal index relating a suite of soils with similar climate, topography and vegetation (Harden,

1982). As such, as long as the soil age has been identified, systematic studies can be implemented across the chronosequence to examine outcomes presumably affected by past management impacts on profile development (Huggett, 1998). Using a chronosequence established in western Kenya, several researchers have found significant fertility trends in agricultural lands converted from forest at different periods over the past century. For example, Ngoze et al. (2008) found that mineral N and plant-available P decreased significantly while P-adsorption increased after 100 years of continuous cultivation. Furthermore, grain yield in older conversions averaged roughly ¼ of that of newer conversions. In follow-up studies, Kimetu et al. (2008) found on the same chronosequence, that the most degraded sites responded to green manures much more positively than those soils from a recent conversion suggesting that inherent fertility has by and large been lost in the older soils of the chronosequence. These studies suggest a mechanical difference in N release patterns with respect to organic sources, which still needs further elucidation.

This study aimed to assess the effect of soil quality as a result of relative age of agricultural conversion on nitrogen mineralization of plant material from a leguminous tree and maize stover. Furthermore, plant residue was mixed with synthetic fertilizer to test any 'priming effect' and any potential interactions with respect to age of soil.

## 4.3 Materials and Methods

### *4.3.1 Soil and Plant Material Characterization*

Soils were gathered at six sub-sample locations and homogenized from the surface 20 cm. at two separate sites along the same chronosequence in western Kenya (34°94'23" E; 00°13'44" N; Kimetu et al., 2008; Kinyangi, 2008; Ngoze et al., 2008). The soils were converted

from Guinneo-Congolean rainforest in 1900 and 2002 and shall be referred to as OLD and NEW, respectively.

Taxonomically, the soils have been classified as humic nitisols - a kaolinite dominated, high clay content soil type (FAO, 1988) and have been cropped to C4 cereals and beans since conversion. The soils differed texturally and chemically as a result, in part, to varying stages of conversion (Table 1).

**Table 1.** Physical and chemical characteristics of soils evaluated in the study.

| Soil | Sand | Silt | Clay | Texture | pH | Organic Matter | Total C | Total N |
|---|---|---|---|---|---|---|---|---|
| | g $kg^{-1}$ soil | | | | | % | | |
| Old | 113.9 | 521.9 | 364.2 | SiCL | 6.28 | 8.65 | 3.06 | 0.24 |
| New | 293.6 | 173.5 | 532.8 | C | 5.77 | 10.97 | 5.71 | 0.50 |

Two soil amendments were used in the current study; FA and maize stover (MA) (Table 2). The FA used in this study was gathered in the Rift Valley of southern Kenya (37° 30' 36"E; 2° 55' 30"S). For this study, only FA leaves were used. MA was cut following senescence on a working field from the chronosequence utilized in this study. Both amendments were air-dried and ground to pass a 2-mm sieve. Soils and amendments were packaged and shipped at ambient temperatures to the Cornell University facilities in Ithaca, NY.

**Table 2.** Chemical composition of residues used to evaluate mineralization.

| Amendment | C | N | C:N | Lignin | Polyphenols | PP+L:N[a] |
|---|---|---|---|---|---|---|
| | % | | | % | | |
| Faidherbia albida | 47.49 | 5.82 | 8.16 | 5.73 | 0.7 | 1.1 |
| Maize Stover | 39.46 | 1.14 | 34.64 | 3.13 | 0.5 | 3.18 |

[a] PP+L = Polyphenolic + Lignin content

Total carbon and nitrogen were determined by dry combustion using a Europa ANCA-GSL CN auto-analyzer (PDZ Europa Ltd., Sandbach, UK). Lignin content was determined by the acid detergent fiber (ADF) method (Anderson and Ingram, 1993). Soluble polyphenolic content was determined using the Folin-Ciocalteu reagent (FC), against a tannic acid standard. The FC is reacted with the filtered extractant from 0.75 g of plant material with 20 mL of a 95% (v/v) methanol mixture followed by 0.7 M $Na_2CO_3$ (Anderson and Ingram, 1993; Ainsworth and Gillespie, 2007). Values were then determined colorimetrically at 765 nm (Hach, Loveland, CO).

### *4.3.2 Soil Preparation and Incubation*

To assay N mineralization, sieved (<2mm), air-dried soils were incubated at 50% water holding capacity at 30 ºC for seven days prior to the start on the experiment. At the start of the trial period, the soils were sampled for initial N values ($T_0$) and then subsequently brought to field capacity based on moisture retention curves determined through the use of pressure plates (Klute, 1986; Danielson and Sutherland, 1986). The soils were then incubated at a constant temperature of 30°C and 99% relative humidity.

The incubation period consisted of five sampling events after the initial sampling, occurring on days 7, 15, 30, 50 and 75. The experimental design was a 2 x 5 factorial arrangement with two soils (Old and New) receiving each amendment: maize stover (MA),

Faidherbia albida (FA), MA + $NH_4SO_4$ (MA Mix), FA + $NH_4SO_4$ (FA Mix) and no amendment (Control). Each treatment was replicated five times for a total of 50 samples.

The amendments were added on an N basis at 100 mg $kg^{-1}$ of N per 75 g of soil. The soil and N amendments (100 mg $kg^{-1}$ amendment-N + soil for a single amendment treatment and 50 mg amendment-N plus 50 mg $NH_4SO_4$-N $kg^{-1}$) were homogenized and sealed in polyethylene bags to be sequentially removed at the designated sample date (i.e., one bag from each treatment for each of the five sample periods). Based on the procedures of Keeney and Nelson (1982), sub-samples were taken in triplicate and analyzed for $NH_4$ and $NO_3$ using a segmented-flow autoanalyzer (Seal Analytical, Mequon, WI) after a 24 hour drying period at 60°C. The extractant was obtained by shaking 8 g soil with 40 ml of 2M KCl on a reciprocal shaker for 30 minutes, then filtered through ashless filter paper (Whatman no. 42).

### *4.3.3 Statistical Analysis*

Net mineralization was measured as the sequential difference in $NH_4$ and $NO_3$ levels between two sample periods. All data were tested for normality and heteroscedasticity and analyzed for statistical difference using the one-way Analysis of Variance (ANOVA) and t-test procedures with the Sigma Plot statistical software package. Means separation was computed using Tukey's HSD.

## 4.4 Results

### *4.4.1. Cumulative Net Mineralization*

The mineralization patterns underscore the inherent fertility differences between the two soils (Table 3, Figure 1). There was a similar mineralization pattern for both soils with an increasing trend over the first 30 days of the incubation, followed by a steady depression period during the next 20 days.

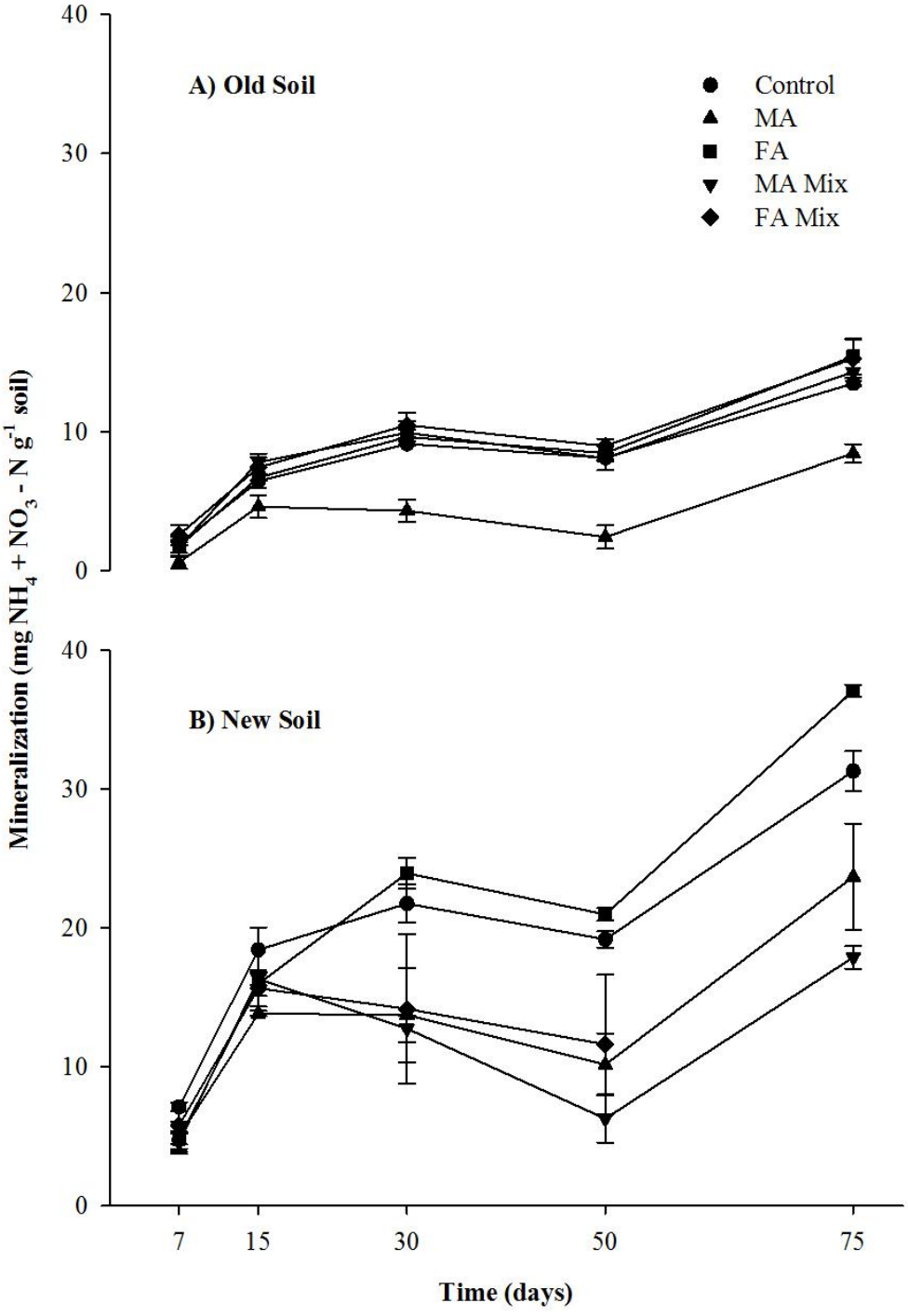


**Figure 1.** Cumulative net mineralization time-series for A) Old soil and B) New soil. Mineralization is calculated as the updated sum of differences between $NH_4$ + $NO_3$ levels at each sample and the initial $NH_4$ + $NO_3$ sample level. Error bars represent the SEM.

Overall fluxes were similar with respect to both immobilization and mineralization. Differences in inorganic N levels of primary mineralization periods, measured during the first two samples and last sample period were both statistically greater in the New soil with a generally increasing trend throughout the incubation (Table 5). Likewise, during the period of greatest immobilization (Day 50), the inorganic N levels were at 81 and 78% of starting values for the Old and New soil respectively.

**Table 3.** Inorganic N production at each sample period. Numbers in parenthesis represent the SEM. Values with different letters represent significant difference at $\alpha = .05$.

| | $NH_4 + NO_3$ - N ($\mu g\ N\ g^{-1}$ soil ) | | | | |
|---|---|---|---|---|---|
| | 7 | 15 | 30 | 50 | 75 |
| Old Control | 7.32 (0.10) | 9.90 (0.80) | 8.09 (0.63)a | 4.38 (0.27) | 10.76 (0.23) |
| Old MA | 5.99 (0.44) | 9.40 (0.53) | 5.11 (0.40)b | 3.51 (0.49) | 11.41 (0.29) |
| Old FA | 7.14 (0.47) | 10.34 (0.61) | 8.32 (0.41)a | 4.24 (0.55) | 12.35 (0.72) |
| Old MA Mix | 7.13 (0.73) | 11.46 (0.66) | 7.51 (0.98)a | 3.57 (0.11) | 11.62 (0.10) |
| Old FA Mix | 7.96 (0.72) | 10.26 (0.46) | 8.43 (0.17)a | 3.91 (0.81) | 11.68 (0.92) |
| New Control | 18.91 (0.32) | 23.11 (1.33) | 15.12 (0.30) | 9.23 (1.95) | 23.91 (1.27) |
| New MA | 16.77 (1.09) | 20.66 (1.21) | 22.73 (3.49) | 8.22 (1.22) | 25.32 (1.59) |
| New FA | 16.61 (0.40) | 22.97 (0.59) | 19.76 (1.01) | 8.83 (0.79) | 27.89 (0.57) |
| New MA Mix | 16.35 (0.81) | 23.58 (0.56) | 8.23 (0.74) | 5.33 (0.91) | 23.41 (1.12) |
| New FA Mix | 17.57 (1.69) | 21.69 (0.56) | 10.32 (4.72) | ND | ND |

$N_i$ production for both FA and MA was more pronounced in the New soil in contrasting ways. By day 75, 3.98% of the added N was mineralized in the FA treatment. New MA, on the other hand, mineralized an average of 2.5% less than the Control over the first 50 days, meaning that there was a net immobilization over this period (Figure 2).

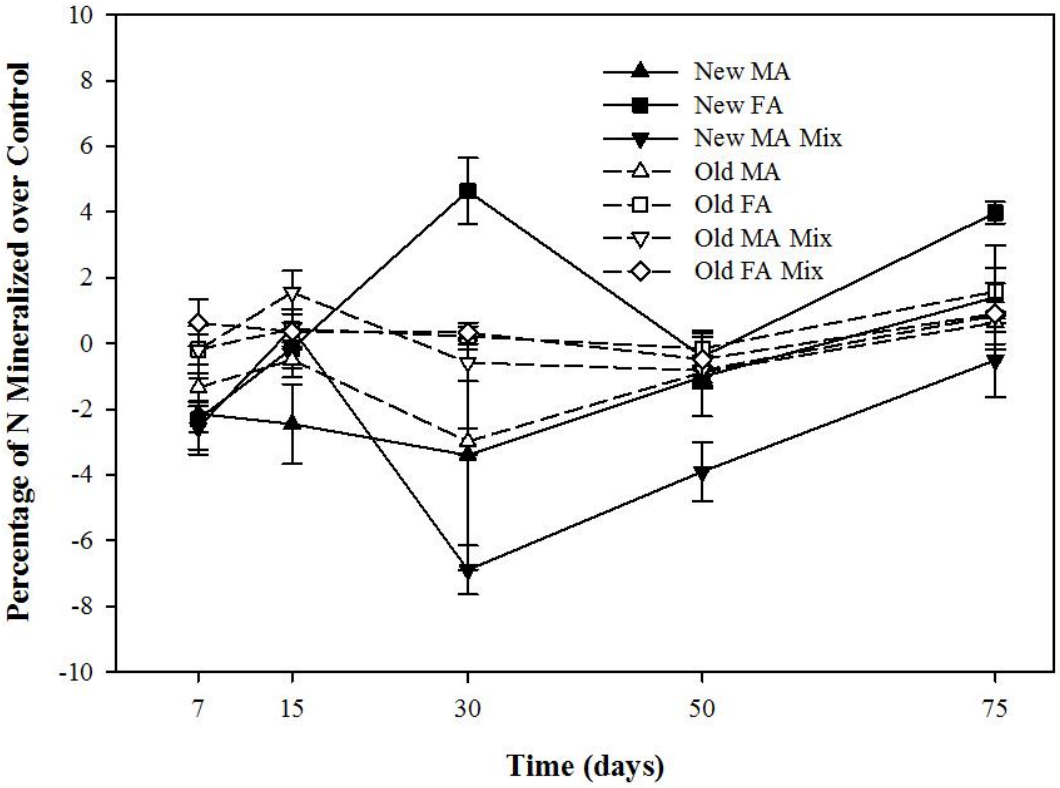


**Figure 2.** N mineralization throughout the 75-day incubation. Percentage of N mineralized is calculated as: ((Mineral N in treated soil – Mineral N in control soil)/ N added to the treated soil)*100 (Vanlauwe et al., 2005). Error bars represent the SEM.

Mixing inorganic fertilizer with organic amendments had very little overall effect. In the New soil MA Mix did result in a net immobilization over the duration of the incubation period. Peak immobilization occurred on day 30, immobilizing 6.89% of added N. The FA Mix

treatment varied very little from the Control treatment and in fact did not show differences in $N_i$ levels between soils where most other amendment treatments did.

**Table 4.** Two-sample t-test comparing differences between soils (New vs. Old). Negative values represent sample periods where values in the Old soil were greater than in the New soil. Symbols indicate levels of significance: * < .05, ** < .01, *** < .001.

| **Sample Period Inorganic N Difference (µg N $g^{-1}$ soil)** | | | | | |
|---|---|---|---|---|---|
| | ***7*** | ***15*** | ***30*** | ***50*** | ***75*** |
| Control | 5.19** | 12.00* | 12.62** | 11.07** | 17.81** |
| MA | 4.38* | 9.23** | 9.44 | 7.74 | 15.25* |
| FA | 3.06 | 9.28** | 14.32** | 12.51*** | 21.64** |
| MA Mix | 2.81 | 8.53** | 2.83 | -1.81 | 3.57 |
| FA Mix | 3.21 | 8.22* | 3.71 | 2.65 | ND |

## 4.5 Discussion

### *4.5.1 Sample Preparation*

To some extent, the mineralization patterns may be a result of preparation effects on the soils and amendments. Specifically, drying and re-wetting of soils and leaves has been found to affect subsequent mineralization patterns. Re-wetting of dried soils, as also occurs at the end of an extended dry season on tropical regions, causes a moderate increase in microbial biomass activity, which may lead to a larger than expected immobilization and/or mineralization rates (Mikha et al., 2005; Xiang et al., 2008). Consequently, a flush of protected, indigenous C may have stimulated mineralization rather than the added amendments thus masking these treatment effects.

### *4.5.2 Base Soils Comparisons*

Mineralization potential was much greater in the New soil as compared to the Old soil, which may in part be contributed to the fact that it had nearly twice the total N at the start of the incubation (0.5% versus 0.24% in the Old soil). Moreover, total C averaged 5.71% in the New soil while C content in the Old soil was 3.06% for a C:N of 11.43 and 12.77 respectively (Table 1). Differences in $N_i$ levels were statistically significant at each sampling period and generally increased throughout the incubation period (Table 5). An immobilization phase was observed between days 30 and 50, which is somewhat anomalous as most of the previous research shows a generally increasing mineralization trend throughout the entire incubation period in the control soils (Palm and Sanchez, 1991; Phombeya, 1999; Sakala et al., 2000).

Finally, during the primary mineralization phase of the incubation (days 50-to-75), the New soil mineralized statistically more $N_i$ averaging 0.59 μg N $g^{-1}$ soil $d^{-1}$ while the Old soil averaged just 0.26 μg N $g^{-1}$ soil $d^{-1}$ during this same period (Table 2). At the end of the incubation, the total available $N_i$ measured was 150% of the starting total N in the Old soil and 151% in the New soil. While overall gains in levels of $N_i$ were similar, the final values in the Old soil were still below starting values in the New soil. Furthermore, the doubling of the mineralization rates during primary mineralization phases corroborates the inherent fertility in the less degraded (New) soil with higher organic matter contents (Ngoze et al., 2008).

### *4.5.3 Effects of Maize and Faidherbia Amendments*

Both soils exhibited a similar pattern with an initial flush of N followed by a period of immobilization. Using pigeonpea (*Cajanus cajan* (L.) Millsp.) and maize stover, Sakala et al. (2000) witnessed a similar release pattern to FA with pigeonpea. Yet in their study, net immobilization persisted for nearly 130 days with a maize residue amendment while in the

present study, the switch from immobilization to mineralization occurred between 50 and 75 days for both soils and for all amendments. The C:N ratio is much lower for the MA amendment in the present study however (35 to 60), which would likely have a positive effect on mineralization patterns.

In general, MA exhibited the expected mineralization pattern of a lower quality amendment, but statistical differences were not detected with respect to the Control soil. Overall, the MA treatment immobilized N throughout the incubation in both soils and was statistically significant after day 30 (Table 5). Because of high variability, the difference between $N_i$ levels was generally not different within soils. However, mineralization rates, particularly in the latter part of the incubation, were higher in the New soil, which could suggest increased activity in the microbial community. Moebius-Clune et al. (2011) found that biologically active carbon declined exponentially under continuous cultivation from newer to older soils along this same chronosequence. This measure was well correlated with overall biological activity.

**Table 5.** Cumulative N mineralization at each sample period. Numbers in parenthesis represent the SEM. Values with different letters represent significant difference at $\alpha = .05$.

| | $NH_4 + NO_3$ - N ($\mu g\ N\ g^{-1}$ soil ) | | | | |
|---|---|---|---|---|---|
| | 7 | 15 | 30 | 50 | 75 |
| Old Control | 1.93 (0.10) | 6.43 (0.48)ab | 9.12 (0.16)a | 8.10 (0.11)a | 13.47 (0.18)a |
| Old MA | 0.59 (0.44) | 4.60 (0.82)b | 4.30 (0.79)b | 2.42 (0.86)b | 8.43 (0.66)b |
| Old FA | 1.75 (0.47) | 6.69 (0.26)ab | 9.61 (0.54)a | 8.45 (0.58)a | 15.41 (1.29)a |
| Old MA Mix | 1.74 (0.73) | 7.80 (0.33)a | 9.91 (0.86)a | 8.08 (0.87)a | 14.31 (0.97)a |

| | | | | | |
|---|---|---|---|---|---|
| Old FA Mix | 2.56 (0.72) | 7.43 (0.98)ab | 10.46 (0.90)a | 8.97 (0.49)a | 15.25 (1.40)a |
| | | | | | |
| New Control | 7.11 (0.32) | 18.42 (1.61) | 21.74 (1.36) | 19.17 (0.59)a | 31.28 (1.44)ab |
| New MA | 4.97 (1.08) | 13.82 (0.33) | 13.74 (3.49) | 10.16 (2.24)b | 23.68 (3.83)bc |
| New FA | 4.81 (0.40) | 15.97 (0.87) | 23.93 (1.07) | 20.96 (0.46)a | 37.05 (0.44)a |
| New MA Mix | 4.55 (0.81) | 16.32 (0.44) | 12.75 (0.95) | 6.27 (1.73)b | 17.88 (0.81)c |
| New FA Mix | 5.77 (1.69) | 15.65 (1.31) | 14.17 (5.37) | ND | ND |

The difference in rates, both mineralization and immobilization, with respect to each amendment may be in part attributable to starting $N_i$ values in the soil. Recous et al. (1995) observed carbon mineralization rates at starting $N_i$ values similar to those in the present Old soil to be approximately half of that of higher starting $N_i$ soils. With smaller $N_i$ pools, the net effect is a slower immobilization per unit of C mineralized. A similar trend appears likely in the immobilization period of the current study, particularly with the lower quality amendment (Figure 1).

For both soils, the effect of FA resulted in greater mineralization compared to the unamended soil, although this was only statistically greater than zero in the New soil (Figure 2). Cumulative mineralization in the latter soil was 5.77 $\mu g\ N\ g^{-1}$ soil greater than the unamended soil. When extrapolated out to an areal basis, this is approximately 10 kg $ha^{-1}$ more $N_i$ in the FA treated soil; significantly lower than values recorded by Phombeya (1999). Based on these studies, it is difficult to translate N dynamics into potential effects on yield. Yield gains are likely complicated by the inherent variability within a field and between soil types as is demonstrated by the present finding that mineralization of FA was more responsive in the New soil (Table 2).

As a percentage of baseline mineralization, MA showed a similar trend in both soils (Figure 2). After 50 days, average mineralization (immobilization) in the MA treatment in the New soil was 2.25% below levels in the Control treatment and -1.42% in the Old soil. Immobilization predominated throughout the incubation, but a mineralization phase appears to begin by day 75 (Figure 2). This trend suggests that that unlike FA, immediate incorporation of MA may be more detrimental to subsequent crop growth, but net benefits may accrue over multiple seasons.

***4.5.4 Fertilizer Mixture***

Interactive effects from the mixture of inorganic and organic amendments were undetected in the present study (Figure 1). We hypothesized that availability of added N in $NH_4SO_4$ would be apparent in higher average initial levels of $N_i$ in these treatments. Overall, the mixed treatments exhibited slightly higher cumulative mineralization than the Control at the end of the incubation in the Old soil but these differences were not significant (Table 4). For the lower quality MA amendment, the fertilizer mixture amplified immobilization rates in the New soil.

Sakala et al. (2000) found similar results incubating a mixture of maize residue and $NH_4$-N with an initial immobilization period followed by net mineralization. Their study demonstrated decreasing immobilization periods with an increase in $NH_4$-N, which is likely the result of the increased inorganic N pool at the start of the incubation. This is reflected only in the Old soil of the present study. These results suggest that adding fertilizer with a lower quality amendment may be more beneficial in more degraded soils. Vanlauwe et al. (2001) stress that while positive results are possible, a complex set of variables exist to confound conclusions on fertility. More research is necessary, however, to determine the temporal differences in mineralization rates as they might compare to crop response.

## 4.6 Conclusion

This study presents data highlighting the inherent differences in mineralization potential between soils at different stages along a chronosequence. High quality amendments will likely have a much greater effect on yield on more degraded soils even though more N may be made available in more fertile soils. These results also suggest that low quality amendments on higher fertility soils may in fact be counter-productive on a short-term basis if applied asynchronous with crop uptake. Furthermore, mixing synthetic fertilizer with organic inputs did not increase available N in any appreciable manner and in fact increased immobilization in the less fertile Old soil.

Overall, FA produced higher average $N_i$ levels although the difference over the control was not statistically significant. When extrapolated out to an areal basis, there was approximately 10 kg $ha^{-1}$ more $N_i$ in the FA treated New soil. When compared to MA, there was nearly 24 kg $ha^{-1}$ more $N_i$ in the FA treated soil. In the Old soil, these differences were approximately 3.5 and 13 kg $ha^{-1}$ respectively. These varying rates may be the result of high N availability in the New soil exerting a stronger influence on C degradation (Recous et al., 2005). Literature reported increased yields found around FA may be the result of more long-term accumulation of FA biomass rather than the result of the most recent application.

This study demonstrates the effect of organic amendments on N availability at two ends of the degradation spectrum. The results also suggest that low quality amendments on higher fertility soils may in fact be counter-productive on a short-term basis if applied asynchronistically with crop uptake. Furthermore, mixing synthetic fertilizer with organic inputs did not increase available N in any appreciable manner and in fact increased immobilization in

the less fertile Old soil. FA showed higher mineralization on average although differences were not significant over the unamended Control.

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